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The Biogeography and Evolution of *Symbiodinium* in Giant Clams (Tridacnidae)

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The Biogeography and Evolution of *Symbiodinium* in Giant Clams (Tridacnidae)

by

Michele Weber

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Doctor of Philosophy

in
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in the
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University of California, Berkeley

Committee in charge:

Jere H. Lipps
Roy L. Caldwell
George K. Roderick

ABSTRACT

The Biogeography and Evolution of *Symbiodinium* in Giant Clams (Tridacnidae)

by
Michele Weber
Doctor of Philosophy in Integrative Biology
University of California, Berkeley
Professor Jere H. Lipps, Chair

Symbiodinium is a diverse lineage of dinoflagellates that forms photoendosymbioses with five phyla of marine invertebrates and protists. The symbiotic relationship provides hosts with a direct source of energetic resources and the dinoflagellates gain access to a safe environment where they can survive in high densities and easily access the raw materials for photosynthesis. In low nutrient, tropical waters, this relationship forms the base of the food web and the reef structure on which diverse communities have evolved.

Although many charismatic reef organisms show clear distribution patterns across their ranges, we are still exploring biogeography with respect to symbiosis. Previously biogeographic patterns in marine microbes were largely ignored; microbes were considered pandemic because lineages would not be limited to particular geographic regions as a result of dispersal and connectivity across oceans. More recently, advanced molecular techniques identified multiple scales of genetic variation in *Symbiodinium* that were not readily apparent from morphological analyses and encouraged investigation of finer scale questions about distributions and evolutionary patterns.

We now know that the genus *Symbiodinium* is diverse and that certain clades are found in unique regions and associate with particular hosts. Distributional data suggests that they were subjected to a selection mosaic and subgeneric clades diverged, geographically and functionally, on various scales but since the data is confined to certain hosts from certain regions, we know little about large-scale patterns. Some host specificity has been observed but since most hosts transmit their symbionts horizontally across generations and symbionts can be exchanged between host lineages, there is limited context for coevolutionary processes to refine particular pairings. I addressed the biogeographic patterns of *Symbiodinium* from giant clams on two different scales: regionally across their Indo West Pacific distribution and locally in the Northern Red Sea. Finally I analyzed the historical biogeography for the holobiont association between giant clams and *Symbiodinium* and inferred historical processes to explain the modern symbiont diversity patterns in a region of low host diversity.

In the first chapter I documented diversity of *Symbiodinium* for two species of giant clam, *Tridacna maxima* and *Tridacna squamosa,* across their Indo West Pacific distribution. At 25 localities across the Indo-Pacific from French Polynesia to the Red Sea, I collected small pieces of mantle tissue and recorded the depth and reef environment where each sample was collected.

A complete distribution of *Symbiodinium* phylotypes in association with giant clams across their range provided a broad indication of what fraction of total *Symbiodinium* diversity associates with this host group. Compared to other host groups, *Tridacna* are specific hosts; of the hundreds of *Symbiodinium* genotypes documented in the literature and on GenBank, only ten distinct types live in giant clams and all had been reported from alternative hosts. Giant clams are crown group metazoans and each generation acquires new symbionts from the environment. While other alternative hosts, such as corals and foraminifera, can transfer symbionts between generations and occasionally evolve lineages of specific symbionts, I did not identify novel clam specific lineages. Giant clams host generalist *Symbiodinium* lineages that are readily available in the water column.

Although their distributional range is similar, *T. squamosa* and *T. maxima* did not host identical symbionts and I observed gradients in symbiont diversity. Symbionts were most diverse in the Central Indo West Pacific and diversity declined in the Pacific and Indian Oceans. While *T. maxima* was a generalist across more of its range and hosted diverse symbionts at most localities, *T. squamosa* was consistently specific for certain symbiont lineages and only in the Central Indo West Pacific did it host more diverse lineages of symbionts. *T. squamosa* lived on deeper reefs than *T. maxima* and the symbionts most often associated with *T. squamosa* exhibited a deeper range than some of the other lineages of *Symbiodinium*. However, *T. maxima* were also collected from deep reefs and they hosted different symbiont lineages. These data showed that multiple lineages of *Symbiodinium* are adapted to depth and they are partitioned between host species. The symbionts were also partitioned between different reef environments. Certain lineages were most common on patch reefs, others on fringing reefs and others from lagoon environments. Phylogenetic systematics indicated that the *T. squamosa* lineage is younger than the *T. maxima* lineage and my data on the distribution of *Symbiodinium* in the two lineages showed that it is also more symbiont specific. This evidence supported the hypothesis that *T. squamosa* is a less obligate host and can more rigorously select for high performing symbionts than *T. maxima*.

In the second chapter I focused on the lack of *Symbiodinium* diversity in *T. maxima* from the Red Sea. Only one phylotype of *Symbiodinium* was identified from *T. maxima* in the Red Sea and these sequences were not found in *Tridacna* samples from other regions. I proposed two hypotheses for the lack of diversity and concluded that multiple symbiont lineages had colonized the Red Sea but only a single lineage was successful and therefore, it replaced the other types and persisted. I compared the Red Sea phylotype to other *Symbiodinium* from alternative hosts in the Red Sea, the Mediterranean Sea and the West Indian Ocean, to show that the most closely related phylotypes exist along the coast of Kenya. I concluded that the Red Sea lineage originated in the West Indian Ocean and colonized the Red Sea via an alternative host that entered through the straits at Bab al Mandab, after the last glacial maximum, 12,000 years ago. Evidence of an endemic holobiont was evaluated with respect to the evolution of cooperation and transitional associations between partners on a geologic time scale. I suggested that the Red Sea phylotype is dominant because it was an infectious lineage. It easily colonized the new host population soon after the Red Sea reflooded, but the endemic holobiont may be transitional and as conditions stabilize, a more cooperative lineage will out-compete and replace the less efficient phylotype.

In the third chapter I addressed a diversity anomaly in the West Indian Ocean. The center of marine biodiversity is the Central Indo West Pacific, which includes Indonesia, the Philippines, Papua New Guinea and the northern Great Barrier Reef in Australia. I sampled

more species of giant clam from Papua New Guinea and Australia and observed more symbiont lineages in association with those hosts than any other region within this study. In the West Indian Ocean I only observed two species of host, as was expected based on the marine biodiversity gradient. However, I also identified five lineages of *Symbiodinium* and while diversity in *T. squamosa* holobionts was lower in the West Indian Ocean, *T. maxima* holobionts were equally diverse in both regions. *T. squamosa* was a generalist in the Central Indo West Pacific and a specialist in the West Indian Ocean but *T. maxima* was a generalist in both regions. I proposed multiple hypotheses to account for these biogeographic patterns including geologic and oceanographic conditions, niche ecology and historical biogeographic patterns for the hosts. Historical biogeography of a holobiont system provides a new framework that includes the history of associations between partners as well as biogeographic patterns for each individual partner. In this case the history of the association between host and symbiont suggested what modern ecology could not explain. I showed that the holobiont range shifted more slowly than the host ranges and that the modern holobiont diversity in the West Indian Ocean is a legacy of Miocene diversity in that region.

For Susan Weber, Lynn Weber and Trent Weber

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The Biogeography and Evolution of *Symbiodinium* in Giant Clams (Tridacnidae)

CHAPTER 1

The distribution of *Symbiodinium* in *Tridacna maxima* and *Tridacna squamosa* across the Indo West Pacific and implications for the evolution of symbiosis in giant clams

ABSTRACT

I documented biogeographic patterns in *Symbiodinium* from *Tridacna maxima* and *Tridacna squamosa* across their Indo West Pacific distributions. *Symbiodinium* from three major clades, A, C and D were identified from the two host species. I evaluated the distribution of *Symbiodinium* in tridacnid hosts with respect to abiotic factors, host specificity, geography and holobiont evolution. Differences in depth range were observed as well as zonation through ecological environments including shallow lagoons, fringing reefs and patch reefs. I observed shallower average depths and more diverse *Symbiodinium* populations in *T. maxima*. *T. squamosa* was found deeper and was primarily specific for two symbiont phylotypes. Subclade level diversity in the two *Tridacna* species could not be independently predicted by host species and abiotic factors alone but both had strong ordering effects on symbiont populations across the Indo-West-Pacific. Certain basal symbiont lineages appeared pandemic across the IWP and other, more derived lineages were concentrated in certain regions or localities suggesting that although multiple types are available to hosts, they may select symbionts based on function. Adaptation to high light habitats and functional autotrophy may force *T. maxima* to host diverse symbionts while the relatively recent divergence of *T. squamosa* and its reversion to heterotrophy allowed the lineage to expand into deeper habitats and associate almost entirely with more derived, less costly symbionts.

INTRODUCTION

Coral reef ecosystems depend on mutualisms between dinoflagellates from the genus *Symbiodinium* and protist or invertebrate hosts. Reef organisms host dinoflagellate symbionts, commonly known as zooxanthellae, because algal photosynthesis provides hosts with additional sources of energy in nutrient poor, tropical seawater (Muscatine and Porter 1977). Algae benefit because they gain access to organic nitrogen, phosphorous and a safe living environment within the tissues of their hosts (Muller-Parker and D'Elia 1997). Ecologically, photosymbiosis is integral because it provides both the energetic basis and the calcium carbonate structure in reef ecosystems (Cowen 1988; Fagerstrom 1988). Research has focused on symbioses in cnidarians and foraminifera and until recently, surprisingly little was known about *Symbiodinium* mutualisms in mollusks.

Investigating the diversity within *Symbiodinium* is an area of active research (Coffroth and Santos 2005). Originally described as a single species, *Symbiodinium microadriacticum* (Freudenthal *sensu strictu*), seemed to be pervasive throughout reef hosts (Freudenthal 1962; Taylor 1973). Eight lettered clades and numerous subclade level lineages of *Symbiodinium* are now recognized from foraminifera, ciliates, poriforans, cnidarians and mollusks (Coffroth and Santos 2005; Pochon et al. 2006; Correa and Baker 2009; Fay et al. 2009). Free-living lineages are also increasingly reported (Hirose et al. 2008; Hansen and Daugbjerg 2009). Additional diversity within morphologically cryptic lineages of *Symbiodinium* is continually revealed, particularly as research focuses on the breakdown of reef symbioses under changing environmental conditions, but it remains unclear if emerging diversity represents a significant majority of the genotypes available to hosts in nature.

Broadly distributed ancestral lineages of *Symbiodinium* form the center of evolving clusters of genotypes (Correa and Baker 2009). These ancestral lineages are common across host taxa and geographic space. For example, *Symbiodinium* ITS2 types C1 and A3 are basal genotypes from which diverse lineages evolved to colonize diverse hosts from all major tropical oceans. A prevailing hypothesis for microbial biogeography is that "Everything is everywhere," meaning prevalent microbes are evenly distributed across landscapes (Martiny et al. 2006) and thus available to hosts. Alternatively, in many host-symbiont systems, uneven selection mosaics create hot and cold spots for the evolution of holobiont diversity across host distributions (Thompson 2005; Hoeksema and Thompson 2007; Thrall et al. 2007). In some regions, hosts and symbionts are part of a tightly locked coevolutionary trajectory. One host species can be specific for one lineage of symbionts in one area and specific for another lineage in another area. Some symbionts may not be specific in all regions and can even be parasitic within a subset of their range or within a different host. If the community is complex or if the environment is highly productive, virulent lineages can evolve and compete with cooperative lineages because there is enough niche space to support cheaters (Klironomos 2003; Denison and Kiers 2004; Sachs and Wilcox 2006; Hoeksema and Thompson 2007). Specific partners may be closely related to generalists or free-living organisms that do not associate as mutualists (Sachs and Simms 2006).

There is little evidence for strict coevolution between *Symbiodinium* and their hosts; host-symbiont specificity varies across the distribution and phylogeny for corals and foraminifera. Because most *Symbiodinium* associate with diverse host taxa, they are not under a specific selection regime controlled by a single host. Soritid foraminifera from Guam hosted multiple lineages of *Symbiodinium* that had never been identified from other hosts suggesting that they

were specific to forams but several common phylotypes often present in corals from other regions were also observed (Pochon et al. 2007). Forams from Guam hosted primarily one lineage of *Symbiodinium* per cell but soritids collected in Papua New Guinea hosted three divergent symbiont lineages per cell (Pochon et al. 2007; Fay et al. 2009). Scleractinian coral dwelling symbionts showed biogeographic patterns between major ocean basins (LaJeunesse 2001; Baker 2003; LaJeunesse 2005; Van Oppen et al. 2005; Goulet et al. 2008). More sampling needs to be done in the boundary zones (i.e.: the Central IWP) to accurately determine where the transitions occur but evidence of geographic, ecological and functional partitioning of symbionts within cnidarians and ciliate hosts demonstrates that symbiont distributions are uneven (Fabricius et al. 2004; LaJeunesse et al. 2004; Loram et al. 2007; Stat et al. 2008; Sampayo et al. 2009). Uneven distributions and variation in specificity indicate that the *Symbiodinium*-host system is subject to a selection mosaic.

Corals and foraminifera are both common *Symbiodinium* hosts but individual species cannot always be distinguished morphologically. Only a few studies document *Symbiodinium* diversity within single host species or lineage across its entire range (for example in octocorals see: Goulet et al. 2008). A more complete sampling scheme addressed *Symbiodinium* communities within diverse hosts but only in regions of intensive research activity, for example, the Great Barrier Reef, of the east coast of Australia or Florida and the Bahamas in the Caribbean Sea (LaJeunesse 2002; LaJeunesse et al. 2004; Van Oppen et al. 2005). Other regions have been sampled sporadically; the Indian Ocean is largely unexplored with the exception of limited studies along the east coast of Africa (Visram and Douglas 2006; Macdonald et al. 2008; Sebastian et al. 2009). The advantage of Tridacnidae as a host system is that all nine species of giant clam are morphologically distinguishable and the most widespread species, *T. maxima* and *T. squamosa,* are distributed across the Indo West Pacific province (IWP) which includes all interconnected marine ecosystems from the tropical Western Pacific Ocean, the Indonesian archipelago, the Indian Ocean and the Red Sea.

Collecting and identifying *Symbiodinium* from across the entire range of host species, including less traditionally studied hosts, will allow me to describe factors that control the selection mosaic in this system and clarify the evolution of this mutualism and symbiont functional diversity across multiple host phyla. I will be able to answer questions about ecological and community diversity, which also have important conservation implications. As climate change raises sea surface temperatures and lows pH, host – *Symbiodinium* relationships disassociate, causing corals to appear bleached and reef mortality around the world (Hughes et al. 2003; Hoegh-Guldberg et al. 2007). Why *Symbiodinium*-host relationships break down under changing conditions is still not well understood. Most of the prevailing hypotheses argue that *Symbiodinium* have adapted to particular abiotic factors and when conditions change, the symbionts cannot perform sufficiently to satisfy the hosts' needs and the association destabilizes (Kinzie et al. 2001; Fitt et al. 2009; Veron et al. 2009).

Tridacnidae appears to be a more resilient host system when faced with environmental change (Leggat et al. 2003). Clams may be sequestering more resilient symbiont lineages or they may be better insulators when conditions are unstable. Under both scenarios they could serve as sinks of available symbionts and conservation of clams may benefit reef communities by providing seeding sources of symbionts as other hosts recover from bleaching events. Understanding biogeographic patterns and identifying which populations host resilient symbionts that may serve as refuges under bleaching conditions, will assist conservationists effectively manage these valuable resources (Hellberg 2007). In this paper I present the distribution and

biogeographic patterns of *Symbiodinium* across the range of the two most common and widespread species of giant clams, *Tridacna maxima* and *Tridacna squamosa*. I identified various lineages of symbionts partitioned across the IWP that are also commonly hosted by the more fragile, reef building, corals and other organisms. This study addresses questions about their relationship to abiotic factors that structure population diversity and presents alternative hypotheses about distribution patterns and holobiont evolution across the IWP.

MATERIALS AND METHODS

Sample collection

Giant clam mantle tissue samples were collected from a variety of depths at 25 sites across the IWP including: French Polynesia, the Cook Islands, Fiji, Vanuatu, New Caledonia, Australia, Papua New Guinea, Thailand, Sri Lanka, the Maldives, Zanzibar, Kenya and Egypt (Figure 1.1). I collected samples from three different reef environments: shallow lagoons, patch reefs and fringing reefs. Lagoons were defined as narrow, less than 1 km from the coastline, and shallow, less than 4m deep. Fringing reefs included forereefs on the outside of the reef crest and reefs along continental coasts where lagoons were not present. Patch reefs were bommies or larger reefs inside of lagoons deeper than 4m where the reef crest was farther than 1km from the coastline. These rough definitions divided the habitat into three bins based on wave energy, flow and proximity to changing temperature regimes and dispersal opportunities (see Appendices A.1.1, A.1.2, A.1.3 and A.1.4 for locality information and environmental data).

At each site, I documented the reef environment and for each individual sampled I recorded host species, depth, length and color morph. To procure the sample, SCUBA divers used a wedge to separate the two valves. Using a hemostat and a pair of nail scissors, I clipped approximately 0.25 grams of *Tridacna* tissue from the mantle. At the surface, the tissue was transferred to tubes containing 80% ethanol and stored at negative 20°C as soon as possible depending on field conditions but not longer than 3 hours.

Sequencing and phylogenetic systematics of *Symbiodinium*

In the laboratory, genomic DNA was extracted from a small portion of the mantle tissue sample using Qiagen extraction kits. The ITS1-5.8S-ITS2 rRNA locus was amplified using primers S_DINO and L_O developed by Pochon et al. (2001), AmpliTaq Gold (Applied Biosystems) and an MJ PTC-200 thermocycler under the following conditions: 94C for 5 min, 40X(94C for 45s, 58C for 45s, 72C for 2 min), 72C for 5 min. The PCR product was quantitated and purified with ExoSAP-IT (USB/Affymetrix) before being sequenced at the UC Berkeley Sequencing Facility with the primer S_DINO.

The electropharograms were reviewed and trimmed in Geneious (Biomatters Ltd.). The sequences were blasted and comparable reference sequences at the clade level were downloaded from GenBank. Using MUSCLE (Edgar 2004), *Symbiodinium* sequences were aligned with appropriate GenBank reference sequences (Table 1.1) from clades A, C and D and trimmed to approximately 730 base pairs. Multiple alignments were run and the iterations confirmed that the majority of the parsimony informative characters were located in ITS2 region. Sequences were trimmed to approximately 300 base pairs and for each clade, a representative of each genotype was aligned with ITS2 sequences from the literature (Table 1.2) representing the common phylotypes (Accession numbers for reference sequences were procured from many

sources including: LaJeunesse 2001; Pawlowski et al. 2001; Pochon et al. 2001; Savage et al. 2002; LaJeunesse 2005; Visram and Douglas 2006; Visram et al. 2006; Correa and Baker 2009; Fay et al. 2009).

MrBayes (Huelsenbeck and Ronquist 2001) was used to infer phylogenetic hypotheses for the genus *Symbiodinium* by aligning the 732 basepairs (for giant clam samples and reference sequences) with the dinoflagellate genus *Gymnodinium* as the outgroup. *Gymnodinium simplex* and *Gymnodinium beii* have been freshly isolated from sea water as free-living dinoflagellates and cultured in the lab (Wilcox 1998). *G. beii* also forms symbiotic associations with pelagic, planktonic foraminifera (Shaked and de Vargas 2006). I also inferred individual hypotheses for relationships within clade A and clade C, using a separate alignment for each clade. The alignments were trimmed to include only the ITS2 locus. To polarize the inference, representative samples from the opposite clade were included as the outgroup. For the phylogenetic analyses, the GTR+I+gamma model for sequence evolution was used and the analyses were each run for 1,500,000 generations. 350,000 generations were discarded as burn-in and the rest were summarized to generate tree topologies and posterior probability values. To confirm phylogenetic hypotheses for the relationships between the major subgeneric clades of *Symbiodinium*, the first ~230 nucleotides from the partial LSU region of the rRNA were trimmed from the sample sequences and then aligned with representative LSU sequences from GenBank (Table 1.3) using MUSCLE and analyzed using MrBayes (Huelsenbeck and Ronquist 2001) as described above. From the four separate analyses of three loci, four trees were drawn: the entire locus and all samples, partial LSU for relationships between the major clades and ITS2 for diversity within clade A and clade C. Representative sequences from each phylotype identified in the *Tridacna* samples were deposited in GenBank.

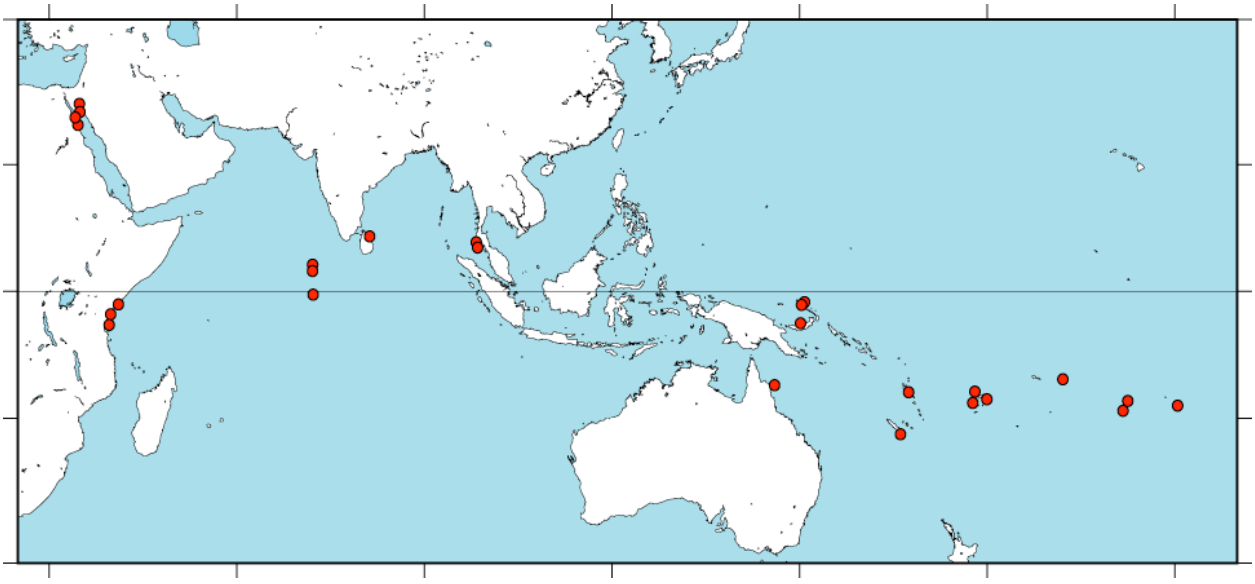

Figure 1.1 Sampling localities

Red spots indicate sampling sites across the Indo West Pacific (IWP). For geographic coordinates see Appendix (A.1.1 and A.1.2).

Clade	Accession	Host	Locality	Reference	
A1	GU068990	*T. maxima*	Egypt	this study	HG.010
A1	GU068984	*T. maxima*	Egypt	this study	RM.009
A3	GU068987	*T. maxima*	Australia	this study	LI.083
A3	GU068985	*T. maxima*	Samoa	this study	OF.009
C1	GU068982	*T. maxima*	Zanzibar	this study	Z.07.237
C1	GU068983	*T. maxima*	Zanzibar	this study	Z.07.170
D1	***	*T.squamosa*	PNG	this study	KV.040
Clade A	AY588453	*Acropora*	Kenya	Visram et al. 2006	
Clade A	AY074949	*Condylactis*	Bermuda	Savage et al. 2002	
Clade A	AJ311946	*Millepora*	Israel	Pochon et al. 2001	
A.med	AY074973	*Anemonia*	Italy	Savage et al. 2002	
A.med	AY588472	*Caryophyllia*	France	Visram et al. 2006	
Clade B	AY074950	*Condylactis*	Bermuda	Savage et al. 2002	
Clade B	AY074966	*Montastraea*	Panama	Savage et al. 2002	
Clade C	AY588462	*Pocillopora*	Kenya	Visram et al. 2006	
Clade C	AF170145	*Pavona*	Australia	unpublished	
Clade C	AJ308893	*Acropora*	Reunion	Pochon et al. 2001	
Clade D	AJ311948	*Acropora*	Guam	Pochon et al. 2001	
Clade D	AJ308900	*Pavona*	Guam	Pochon et al. 2001	
Clade F	AJ311945	*Amphisorus*	Israel	Pochon et al. 2001	
Clade F	AJ291525	*Marginopora*	Guam	Pawlowski et al. 2001	
Clade G	AJ291537	*Amphisorus*	Guam	Pawlowski et al. 2001	
Gymnodinium beii	DQ195345	*Orbulina*	Atlantic (pelagic)	Shaked and de Vargas et al. 2006	
Gymnodinium beii	DQ195374	*Orbulina*	Atlantic (pelagic)	Shaked and de Vargas et al. 2006	

Table 1.1
Sequences including the loci ITS1-5.8S-ITS2-partial LSU referenced in the literature were downloaded from GenBank and aligned with sequences from this study to to identify *Symbiodinium* phylotypes (see Figure 1.2). Samples from this study are in the top half of the table and stars indicate that the sequences have not yet but will be uploaded to GenBank.

ITS2 type	Accession	Host	Locality	Reference	Sample
A1	***	*T. maxima*	Egypt	this study	HG.012
A3	GU068986	*T. maxima*	Moorea	this study	NS.002
A3	GU068987	*T. maxima*	Australia	this study	LI.083
A3a	***	*T. maxima*	Maldives	this study	AD.003
A3x	GU068985	*T. maxima*	Samoa	this study	OF.002
A4	***	*T. maxima*	Kenya	this study	LU.012
A6	***	*T. maxima*	Moorea	this study	ECB.002
C1	***	*T. squamosa*	Fiji	this study	KO.027
C1	GU068983	*T. maxima*	Zanzibar	this study	Z.07.170
C2	***	*T. maxima*	PNG	this study	KB.035
C66	***	*T. crocea*	Australia	Chapter 3	LI.021
D1	***	*T. squamosa*	PNG	this study	KV.040
A1	AF333505	*Cassiopeia*	culture	LaJeunesse 2001	
A2	AF333506	*Zoanthus*	culture	LaJeunesse 2001	
A3	AF333507	*Hippopus*	culture	LaJeunesse 2001	
A3a	EU449035	*unknown*	culture	unpublished	
A4	AF333509	*Plexaura*	culture	LaJeunesse 2001	
A5	AF333508	*T. squamosa*	culture	LaJeunesse 2001	
A6	EU449036	*unknown*	culture	unpublished	
C1	EU786002	*Amphisorus*	PNG	Fay et al. 2009	
C1	AF333515	*Rhodactis*	culture	LaJeunesse 2001	
C2	AF333518	*Hippopus*	culture	LaJeunesse 2001	
C66	AB294633	*Corculum*	Japan	unpublished	
C66	AY589771	*Porites*	E. Pacific	LaJeunesse 2005	
Clade B	AF333511	*Aiptasia*	culture	LaJeunesse 2001	
Clade D	AJ311948	*Acropora*	Guam	Pochon et al. 2001	
Clade F	EU786036	*Amphisorus*	PNG	Fay et al. 2009	
Clade H	EU786028	*Amphisorus*	PNG	Fay et al. 2009	

Table 1.2
To identify fine scale diversity, *Symbiodinium* ITS2 sequences were downloaded from GenBank were analyzed with the ITS2 sequences in this study. (see Figures 1.4 and 1.5). Samples from this study are in the top half of the table and stars indicate that the sequences have not yet but will be uploaded to GenBank.

Clade	Accession	Host	Locality	Reference	
A1	GU068990	*T. maxima*	Egypt	this study	HG.010
A1	GU068984	*T. maxima*	Egypt	this study	RM.009
A3	GU068987	*T. maxima*	Australia	this study	LI.083
A3	GU068985	*T. maxima*	Samoa	this study	OF.009
C1	GU068982	*T. maxima*	Zanzibar	this study	Z.07.237
C1	GU068983	*T. maxima*	Zanzibar	this study	Z.07.170
D1	***	*T.squamosa*	PNG	this study	KV.040
Clade A	AY588453	*Acropora*	Kenya	Visram et al. 2006	
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A.med	AY588472	*Caryophyllia*	France	Visram et al. 2006	
Clade B	AY074950	*Condylactis*	Bermuda	Savage et al. 2002	
Clade B	AY074966	*Montastraea*	Panama	Savage et al. 2002	
Clade C	AY588462	*Pocillopora*	Kenya	Visram et al. 2006	
Clade C	AF170145	*Pavona*	Australia	unpublished	
Clade C	AJ308893	*Acropora*	Reunion	Pochon et al. 2001	
Clade D	AJ311948	*Acropora*	Guam	Pochon et al. 2001	
Clade D	AJ308900	*Pavona*	Guam	Pochon et al. 2001	
Clade F	AJ311945	*Amphisorus*	Israel	Pochon et al. 2001	
Clade F	AJ291525	*Marginopora*	Guam	Pawlowski et al. 2001	
Clade G	AJ291537	*Amphisorus*	Guam	Pawlowski et al. 2001	
Gymnodinium beii	AF060900	free-living	culture	Wilcox 1998	
Gymnodinium simplex	AF060901	free-living	culture	Wilcox 1998	

Table 1.3
To identify evolutionary relationships between the major clades, *Symbiodinium* Ribosomal Large Subunit (LSU) sequences downloaded from GenBank were analyzed with the sequences in this study. (see Figure 1.3). Samples from this study are in the top half of the table and stars indicate that the sequences have not yet but will be uploaded to GenBank.

Statistics

A Chi square test was used to identify different depth ranges for each species and for the *Symbiodinium* phylotypes hosted by *T. maxima* and *T. squamosa* populations across the IWP. I used ANOVA to compare depth distribution for the seven most common symbiont phylotypes (excluding rare symbiont types) for all host species and separately for *T. maxima* and *T. squamosa*. I also investigated the ability of a logistic regression model to predict symbiont phylotype using the depth for each samples. As a proxy for additional variables I could not independently test, I evaluated symbiont distribution in shallow lagoons, patch reefs and fringing reefs. I explored the relationship between ITS2 types and reef environment as categorical variables using a contingency analysis to determine significance of partitioning and correspondence analysis to determine relationships between the symbiont lineages and categorical environmental variable. Testing for relationships between symbiont type and habitat also incorporated the synergistic effects of these combined factors. Therefore, the habitat analyses were not independent from tests for depth but they represented a more complex series of interacting factors. All statistical tests were performed in Microsoft Excel and JMP 8 (SAS Institute Inc.).

RESULTS

Phylogenetics and ITS2 types

Tridacna maxima hosted *Symbiodinium* from clades A, C and D. Phylogenetic analysis of the entire locus resolved some relationships within each clade but inter clade relationships could not be distinguished because the spacer regions were very divergent (Figure 1.2). Analysis of the first ~230 base pairs in the more conserved partial LSU portion of the locus revealed evolutionary relationships between the major clades that were consistent with reconstructions from the literature (Pochon et al. 2006). Clade A was basal to the rest of the tree; it was the first to diverge from free-living ancestors. Clade D was nested within the tree and Clade A phylotypes formed a diverse crown group (Figure 1.3). Many of the phylotypes I identified within these major clades were common lineages across the IWP. The ITS2 locus revealed additional diversity within each of the major clades and all of the symbiont ITS2 types recovered from this study had been previously sequenced from other host taxa and so I followed the conventional naming system which included a letter to designate clade within *Symbiodinium* and a number to indicate subclade level lineage diversity within the ITS2 locus. A second, lower case letter after the number was used to specify a particular lineage having presumably diversified from an ancestral ITS2 type (LaJeunesse 2005; Correa and Baker 2009). Within clade A, I used reference sequences from the literature to identify six unique lineages including the two ancestral A lineages, A1 and A3 that have been reported from diverse hosts around the world (Correa and Baker 2009; LaJeunesse et al. 2009). I also identified ITS2 types A3a, A6, A4 and another well resolved but previously unnamed ITS2 type was designated A3x because it was closely related to A3 and A3a (Figure 1.4). The ancestral types A1 and A3 were 95.7% similar across the ~730 nucleotide rRNA locus. ITS2 types A3a, A3x and A6 were closely related lineages, derived from A3 and differing from the ancestral A3 sequence by a single nucleotide within the ITS2 region. A4 was represented by a single sequence and was most closely related to A1; it differed from A1 sequences by 4 nucleotide positions within ITS2 and approximately 3% over the entire locus. The percent genetic similarity data are summarized in Table 1.4. Within clade C, I identified three unique lineages, C1, C2 and C66. ITS2 types C1 and C2 were 97.8% similar across the entire locus and C66 was 2% divergent from C1. Phylotype D1 was approximately 60% similar to the clade A types and 65% similar to the clade C types. The phylogenetic relationships between these lineages within the *Symbiodinium* crown group are shown in Figure 1.5.

Out of 163 *T. maxima* individuals, 121 hosted Clade A symbionts, 39 hosted clade C symbionts and 3 hosted clade D symbionts. Several subclade level types were identified within each lineage: phylotypes A1, A3 and C1 were common and A3a, A3x, A4, A6, C1, C2, C66 and D1 were sequenced from at least one individual. I sequenced *Symbiodium* from 68 *T. squamosa* individuals. Nine hosted symbionts from clade A, including A1, A3, A3a and A3x. The majority hosted clade C; 46 individuals from across the distribution hosted C1. Clade D was also common and 13 *T. squamosa* hosted symbionts from ITS2 type D1. No ITS2 types were specific to *T. squamosa*. ITS2 type A6 was common in *T. maxima* but not present in *T. squamosa* and one example of A4, one of C66 and two of C2 were also found in *T. maxima* but not *T. squamosa*. The data are summarized in Table 1.5 and Table 1.6 but for individual samples from each host species and their symbiont phylotypes see the Appendix.

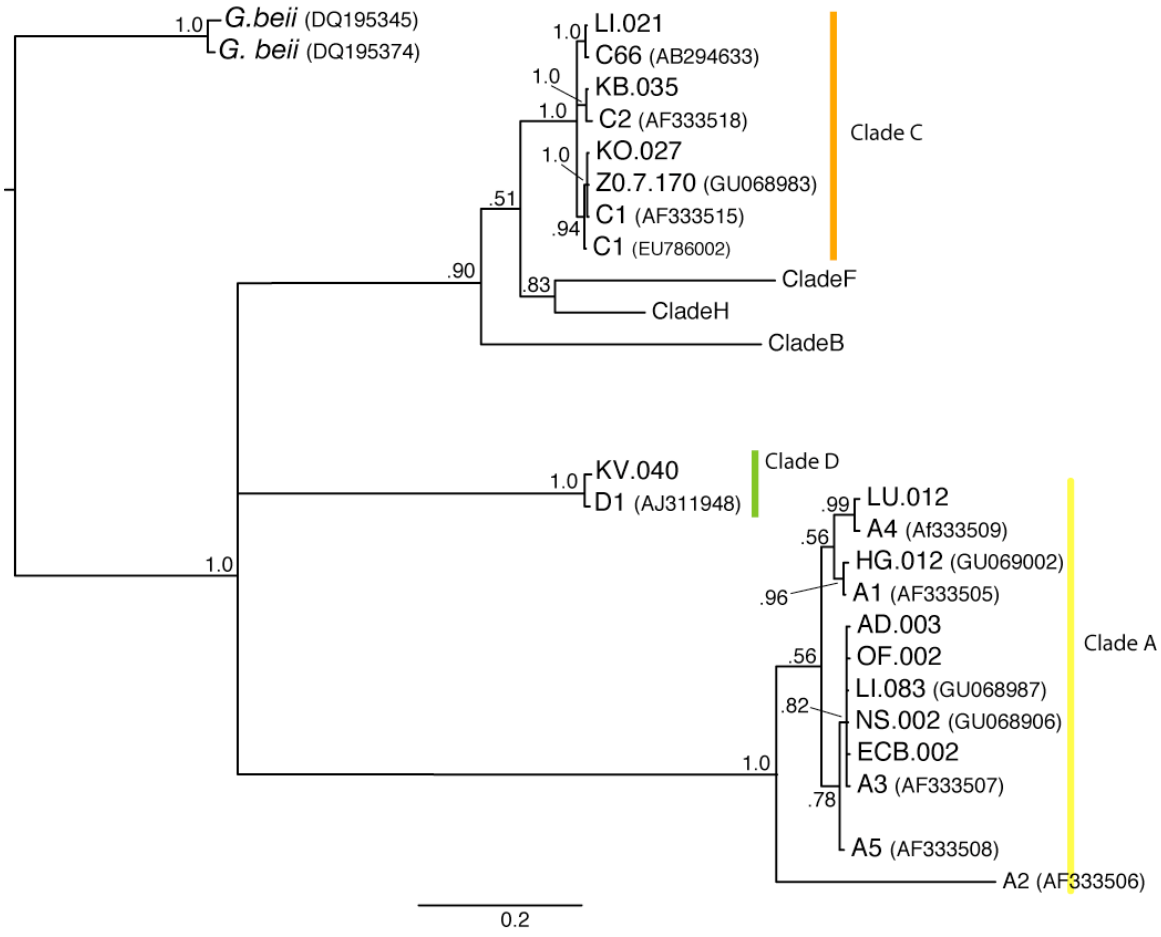


Figure 1.2 Phylogenetic systematics for *Symbiodinium* from *Tridacna*

Evolutionary hypothesis for relationships within *Symbiodinium* sampled from giant clams. Clams hosted symbionts from clades A (yellow), C (orange) and D (green). Phylogenetic inference was based on 732 nucleotide positions including ITS1-5.8S-ITS2-partial LSU in the rRNA. Branch support values are Bayesian posterior probability values. The free-living species *Gymnodinium beii* was used as an outgroup and because of substantial divergence within the noncoding spacer regions, the analysis could not resolve relationships between the major clades and nodes were collapsed.

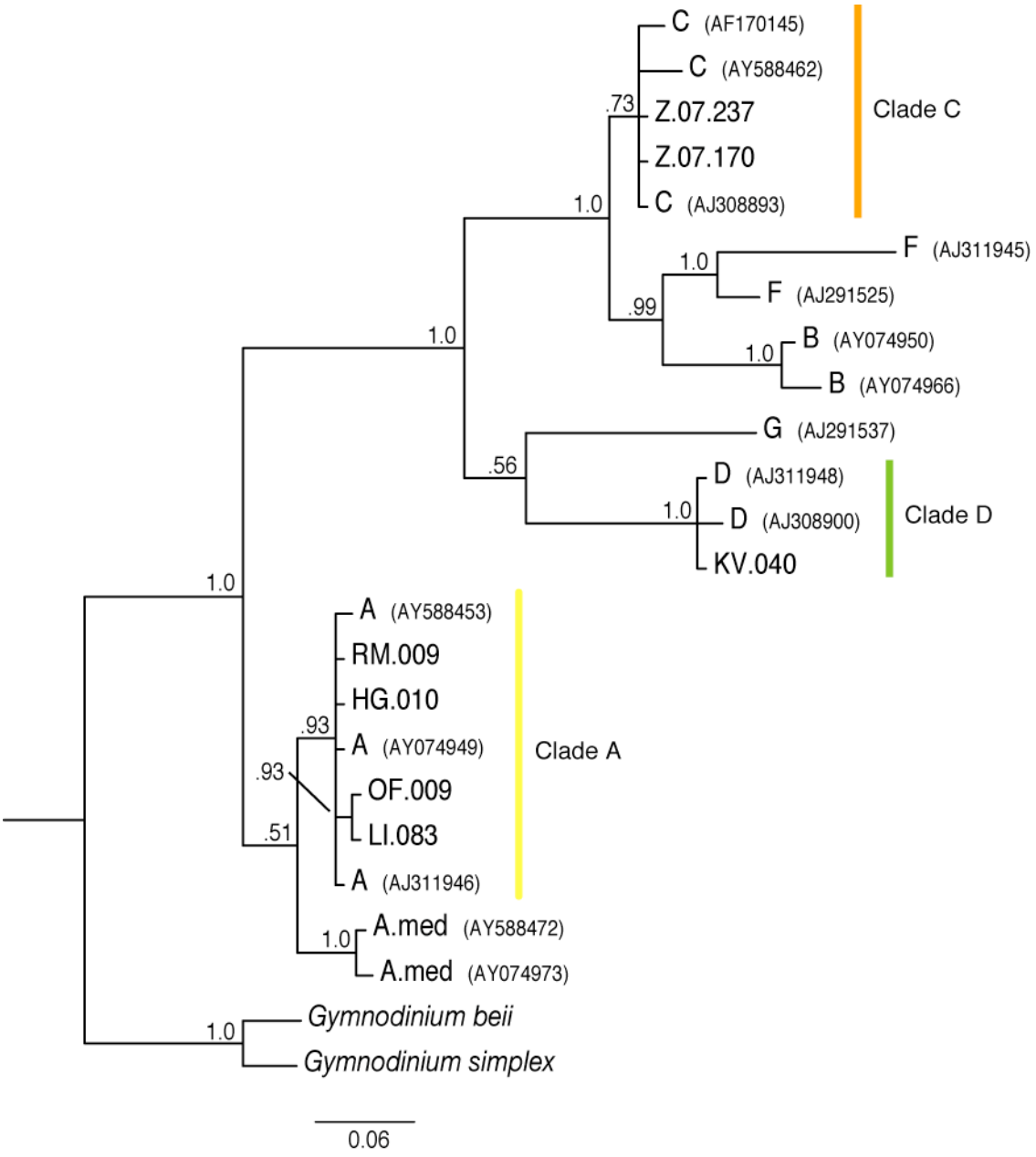


Figure 1.3 Phylogenetic systematics for *Symbiodinium* from *Tridacna*

Phylogenetic inference using the first ~230 nucleotides in the LSU locus resolved inter clade relationships for the genus *Symbiodinium*. Non-symbiotic species *Gymnodinium beii* and *Gymnodinium simplex* were used as outgroup taxa. Branch support values are Bayesian posterior probability values. Within *Symbiodinium,* clade A (yellow) was basal and most closely related to the free-living taxa and clade C (orange) along with clades F, G and H formed the crown group.

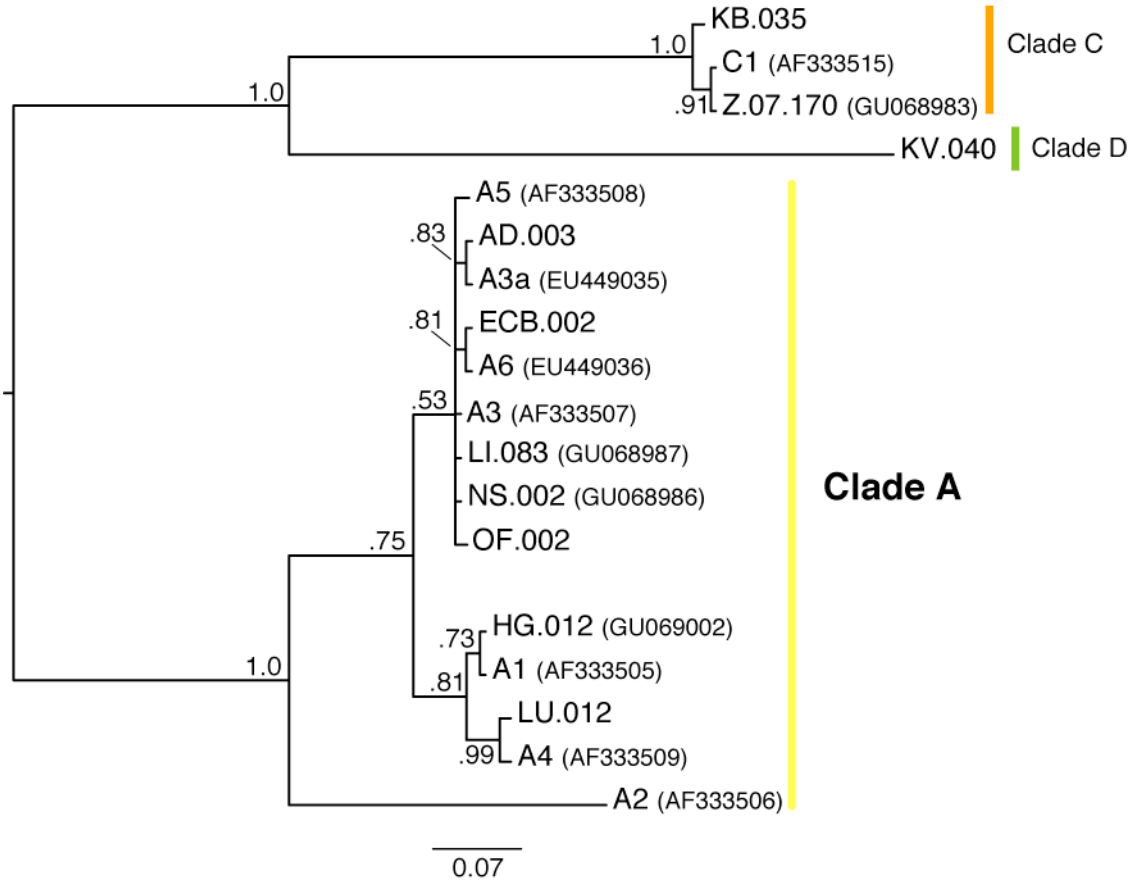


Figure 1.4 ***Symbiodinium*** **phylotypes within Clade A**

Phylogenetic hypothesis for the relationships within clade A; included 304 base pairs from the ITS2 locus for representative samples. Samples from clade C (orange) were used as outgroup taxa. Branch support indicated at the nodes are Bayesian posterior probability values. The novel phylotype A3x was represented by sample OF.002 from Samoa.

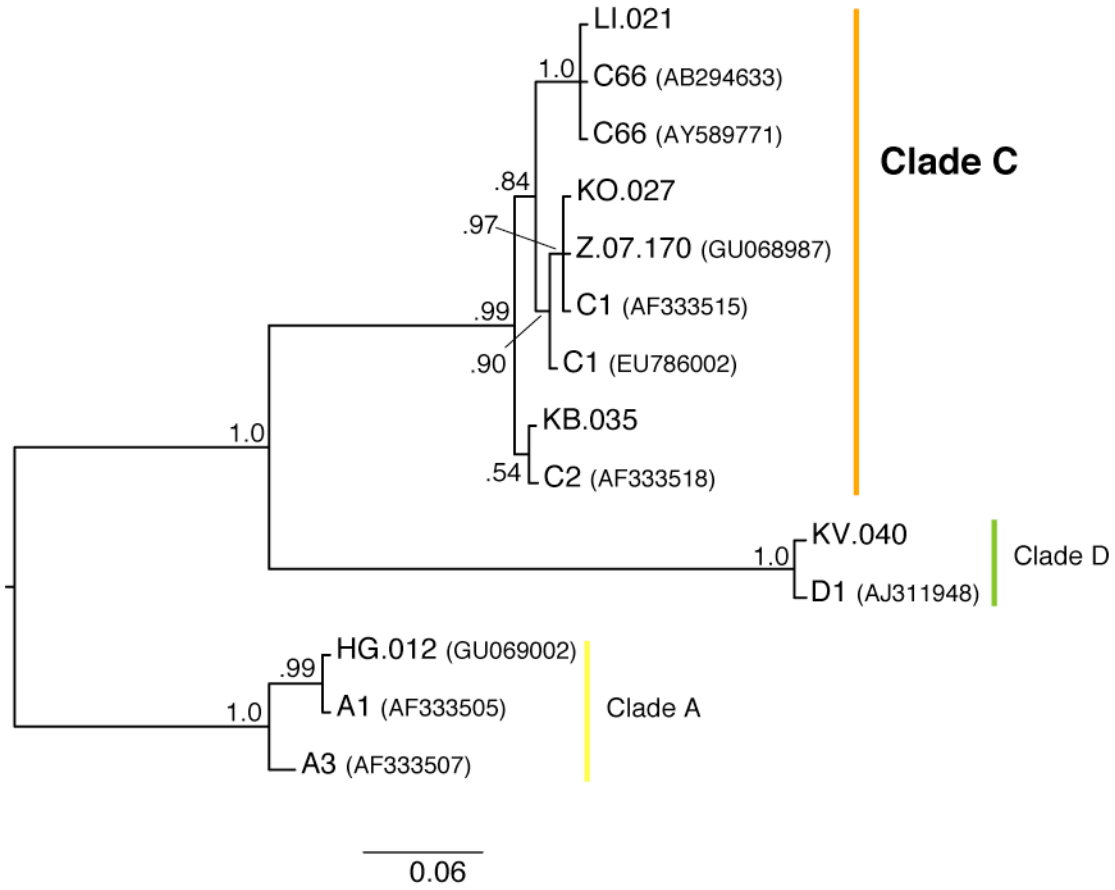
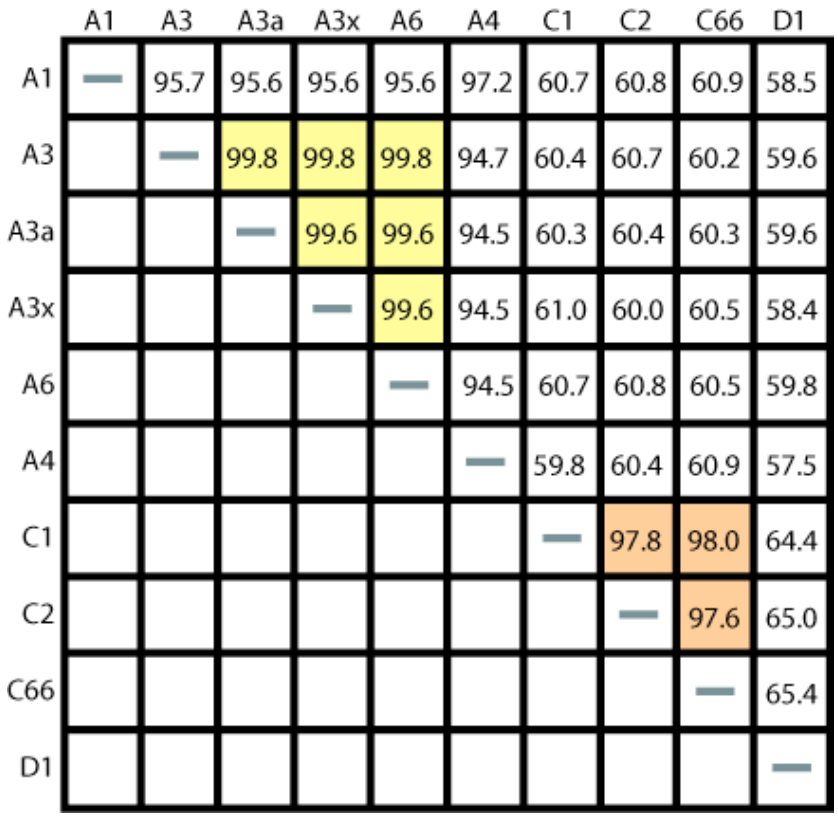


Figure 1.5 ***Symbiodinium*** **phylotypes within Clade C**

The phylogenetic hypothesis for the relationships within clade C included 315 base pairs from the ITS2 locus for representative samples. Samples from clade A (yellow) and clade D (green) were used as outgroup taxa. Branch support values indicated at each node are Bayesian posterior probabilities.



	A1	A3	A3a	A3x	A6	A4	C1	C2	C66	D1
A1	—	95.7	95.6	95.6	95.6	97.2	60.7	60.8	60.9	58.5
A3		—	99.8	99.8	99.8	94.7	60.4	60.7	60.2	59.6
A3a			—	99.6	99.6	94.5	60.3	60.4	60.3	59.6
A3x				—	99.6	94.5	61.0	60.0	60.5	58.4
A6					—	94.5	60.7	60.8	60.5	59.8
A4						—	59.8	60.4	60.9	57.5
C1							—	97.8	98.0	64.4
C2								—	97.6	65.0
C66									—	65.4
D1										—

Table 1.4
Percent similarity for ~730 bp of the ITS1-5.8S-ITS2-partial LSU locus. The 4 closely related lineages within clade A are yellow and the 3 types within clade C are orange (Figures 1.2 and 1.3).

Depth

Although I collected samples from a similar range of depths (from 0 to 25m) for both species, *T. squamosa* was found deeper than *T. maxima*. The mean depth distribution of *T. squamosa* was 10.5m with a standard error of 0.6m and the average depth for *T. maxima* was 5m with a standard error of 0.4m. The results of the t test, assuming equal variance were significant ($p < .0001$) and showed distinct depth ranges for the two clam species (Figure 1.6). I also tested the distribution of symbiont types by depth and rejected the null hypothesis that the most common symbiont ITS2 types had indistinguishable depth ranges. The mean depth ranges for A3, A3x and A6 was between 3-4 meters. The mean depth for phylotypes A3a, C1 and D1 was approximately 8m and the mean depth for ITS2 type A1 was 10m (Figure 1.7). These results were statistically significant (ANOVA, $p < .001$). An independent ANOVA on the symbiont depth distributions for *T. maxima* alone was also significant ($p <.001$) and similar to the total data test but the mean depth for phylotype C1 in *T. maxima* was less, approximately 5 m (not shown). The individual test of *T. squamosa* alone was not statistically significant ($p=.57$); the depth ranges of ITS2 types C1 and D1 were statistically indistinguishable (see Appendices A.1.2 and A.1.4 for depth data). A logistic regression model could not predict symbiont phylotype based on depth for either individual species or both together ($r^2 = 0.07$).

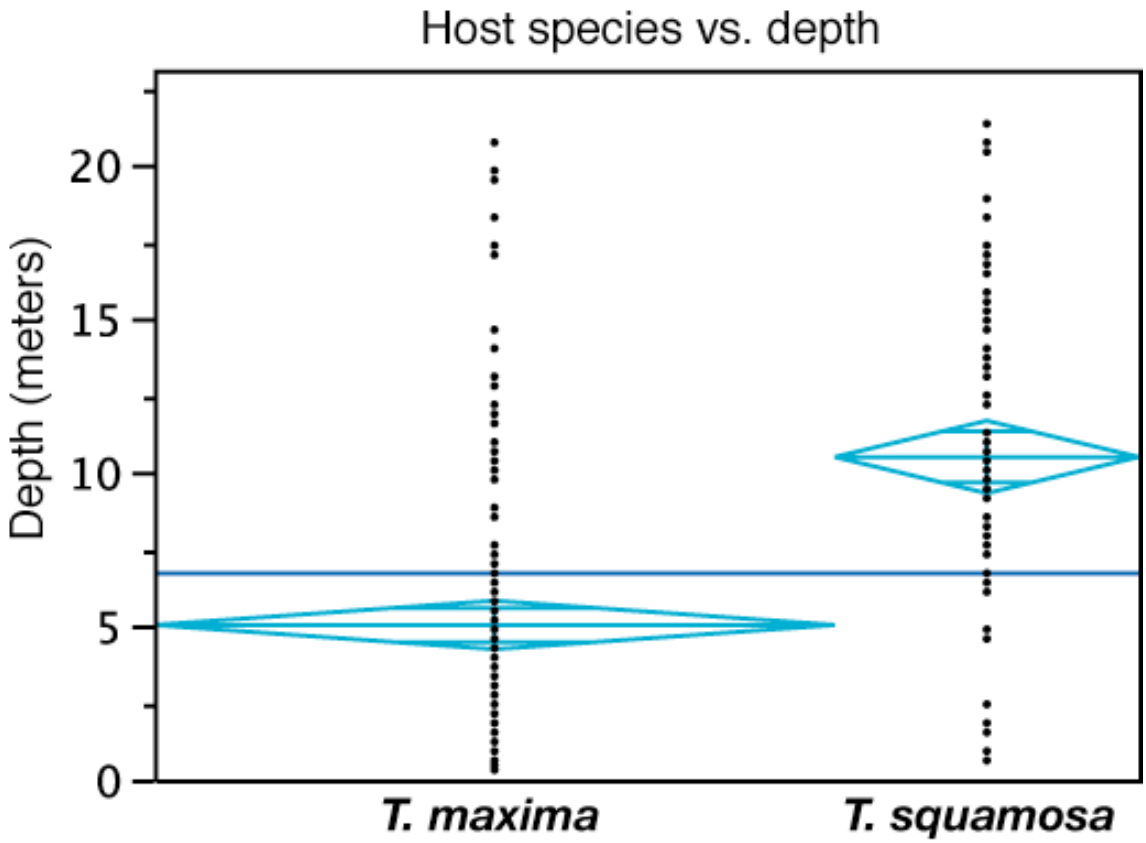


Figure 1.6 Depth ranges for *T. maxima* and *T. squamosa*

T. squamosa lived deeper than *T. maxima* (ANOVA p<.0001). The average depth for *T. maxima* was 5m and the average depth for *T. squamosa* was 11m. The width of the diamonds is proportional to the number of samples for each species and the blue line at 7 meters is the grand mean for all samples collected.

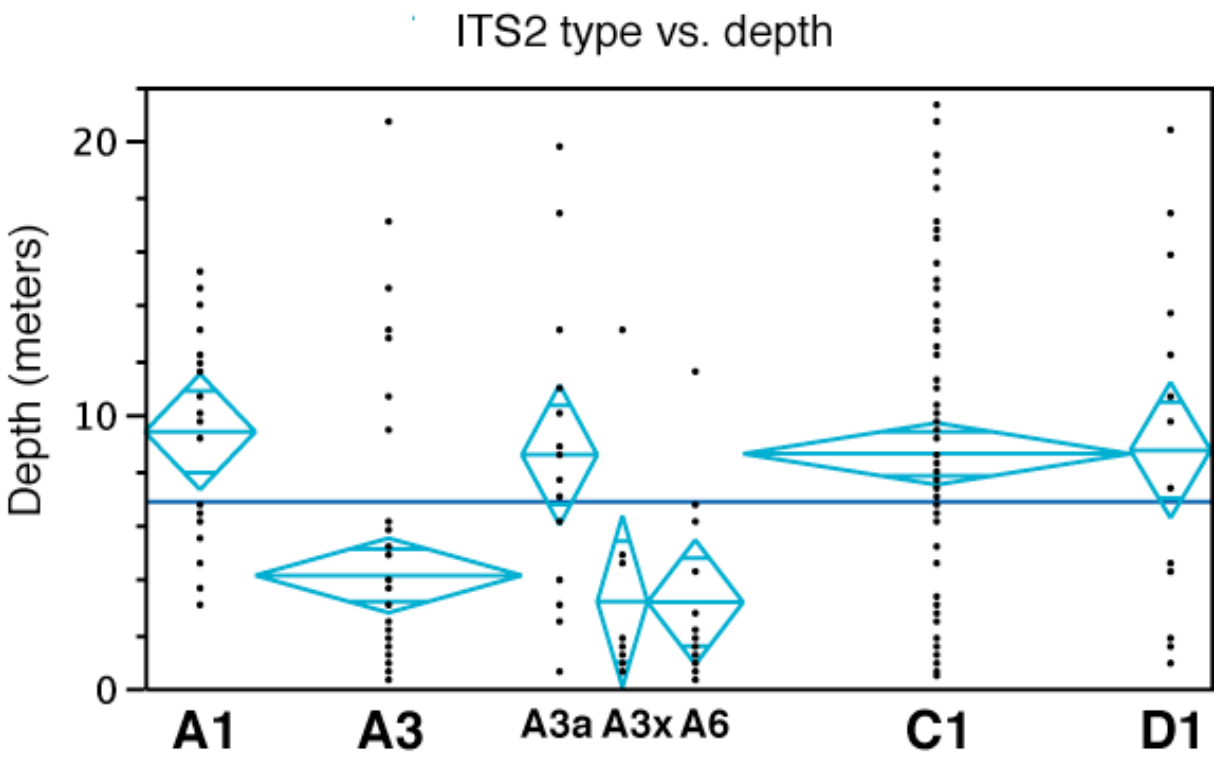


Figure 1.7 Depth ranges for ITS2 types

Symbiodinium ITS2 types were partitioned by depth rejecting the null hypothesis that all symbionts were equally likely to be sampled from all depths (ANOVA, $p<.001$). The width of the diamonds is proportional to the number of samples for each phylotype and the blue line at 6.6 meters is the grand mean for all samples included in this test. Phylotypes A3, A3a, A3x and A6 were more likely to be sampled from shallow reefs and phylotypes A1, A3a, C1 and D1 were more likely to be sampled from deeper reefs.

Reef environment

Contingency analysis suggested that the six most common ITS2 types were partitioned by reef environment. The Chi square test was statistically significant (X2=81.811; p<.0001) rejecting the null hypothesis that the ITS2 types are evenly distributed between the three reef environments (Figure 1.8; Table 1.5). Correspondence analysis using symbiont phylotypes identified from both *T. maxima* and *T. squamosa*, ordinated the reef environments and ITS2 types by two principal coordinate axes. ITS2 types A3 and A6 were associated with lagoon environments and that A1 was more common on fringing reefs. Phylotype D1 symbionts were more common on patch reefs and ITS2 types C1 and A3a were associated with both fringing reefs and patch reefs (Figure 1.9).

I was unable to correlate symbiont type with clam color morphs. A wide variety of color morphs were observed ranging from brown to green to gold to blue and purple but symbiont phylotypes were not related to these morphological differences. Each species exhibited a characteristic colors and patterns. *T. squamosa* was less variable than *T. maxima* but for neither species, did color appear to be distributed by depth or reef environment. Length measurements were normally distributed within each of the two species and did not appear to have an effect on the symbionts (data not shown).

Specificity

Although most symbiont phylotypes were identified in both host species, contingency analysis showed that the seven most common ITS2 types were distributed differently within the two host species. A Chi square test was statistically significant (X^2=76.184; p<.0001) and therefore rejected the null hypothesis that the symbiont phylotypes were evenly distributed between the two host species (Figure 1.10; Table 1.6). Phylotype A3 was the most common symbiont in *T. maxima* populations and it dominated everywhere except the Red Sea. Out of 163 *T. maxima* individuals, 31% hosted A3 symbionts and 60% hosted A3 or a phylotype differing by only 1 nucleotide within the ITS2 locus. Of the remaining 40 % of *T. maxima* individuals, 21% hosted phylotype C1 and the remaining 19% hosted minority lineages. Symbionts from *T. squamosa* populations were more specific than *T. maxima* symbionts. Phylotype C1 dominated in *T. squamosa* across its range; 67% of sampled individuals. Clade D was also common and of the present in 19% of individuals and the remaining 14% hosted other minority phylotypes (data summarized in Table 1.6 but also see the Appendix). A Chi square test was statistically significant for these results (p<.0001) rejecting the hypothesis that the symbionts were evenly distributed between the two host species.

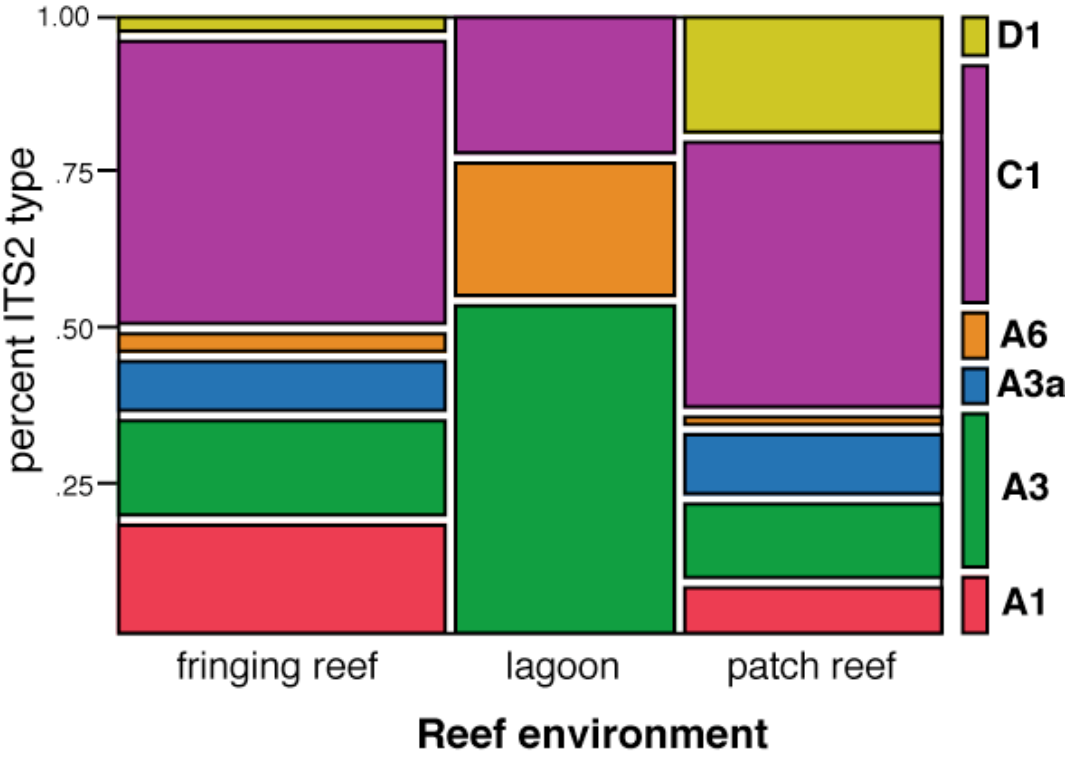


Figure 1.8 Mosaic plot of ITS2 types and reef environments

Symbiont phylotypes (both species combined) were unevenly distributed between three reef environments, patch reefs, fringe reefs and lagoons (X^2=81.811; p<.0001). Width of the columns is proportional to number of samples from each environment and the Y-axis represents the proportion of each phylotype identified from each environment. See Table 1.5.

	A1	A3	A3a	A6	C1	D1
Fringing reef	16	14	8	4	39	3
	7.73	6.76	3.86	1.93	18.84	1.45
Lagoon	0	31	0	13	13	0
	0	14.98	0	6.28	6.28	0
Patch reef	6	9	7	2	29	13
	9.09	4.35	3.38	0.97	14.01	6.28

Table 1.5
Contingency table used to calculate the Chi squared statistic to test the null hypothesis that the most common symbiont phylotypes were equally partitioned between three different reef environments. The top row for each reef description is the number of individuals hosting each symbiont phylotype and the bottom row is the percent of the total (X^2=76.184; p<.0001 for 217 host individuals analyzed).

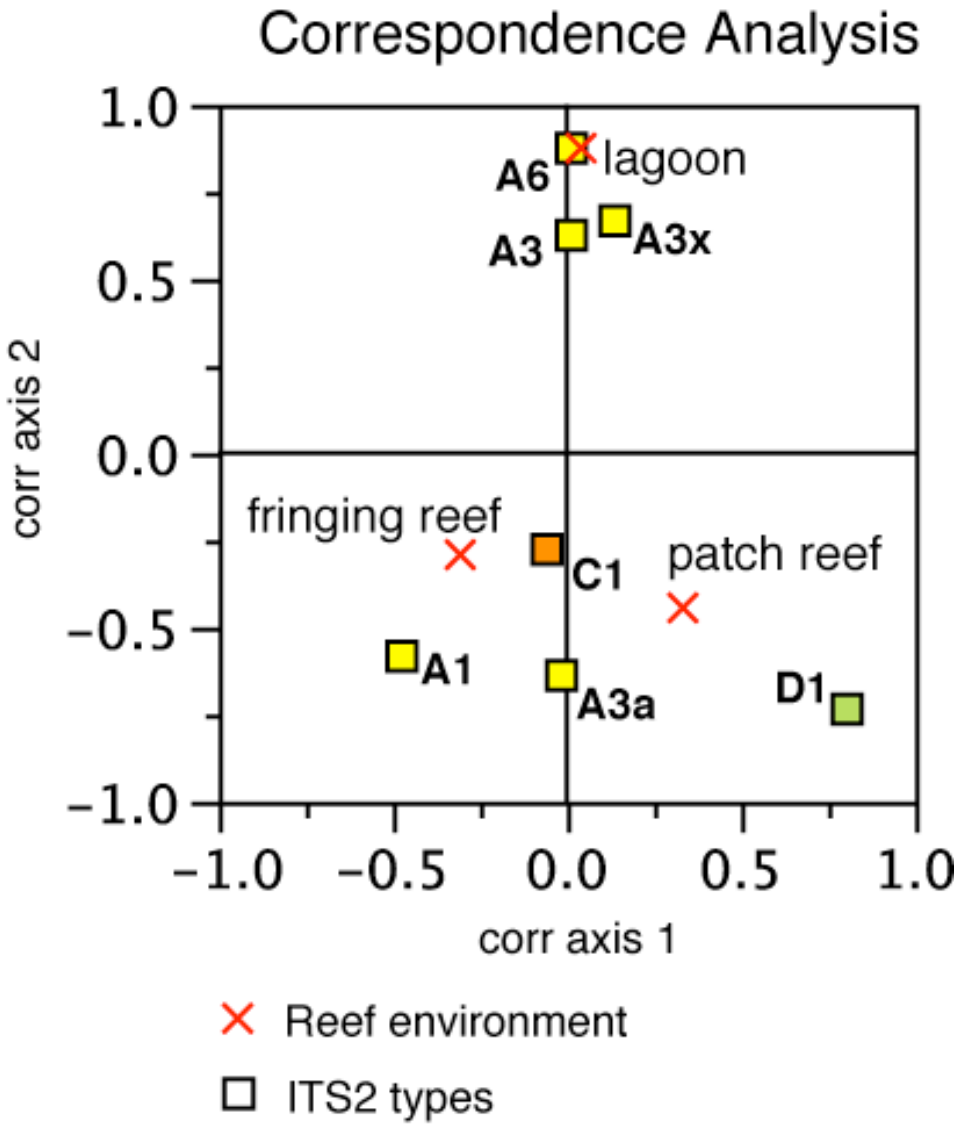


Figure 1.9 ITS2 types partitioned by reef environment

Correspondence analysis was used to visualize the association between the common symbiont phylotypes and three reef environments.

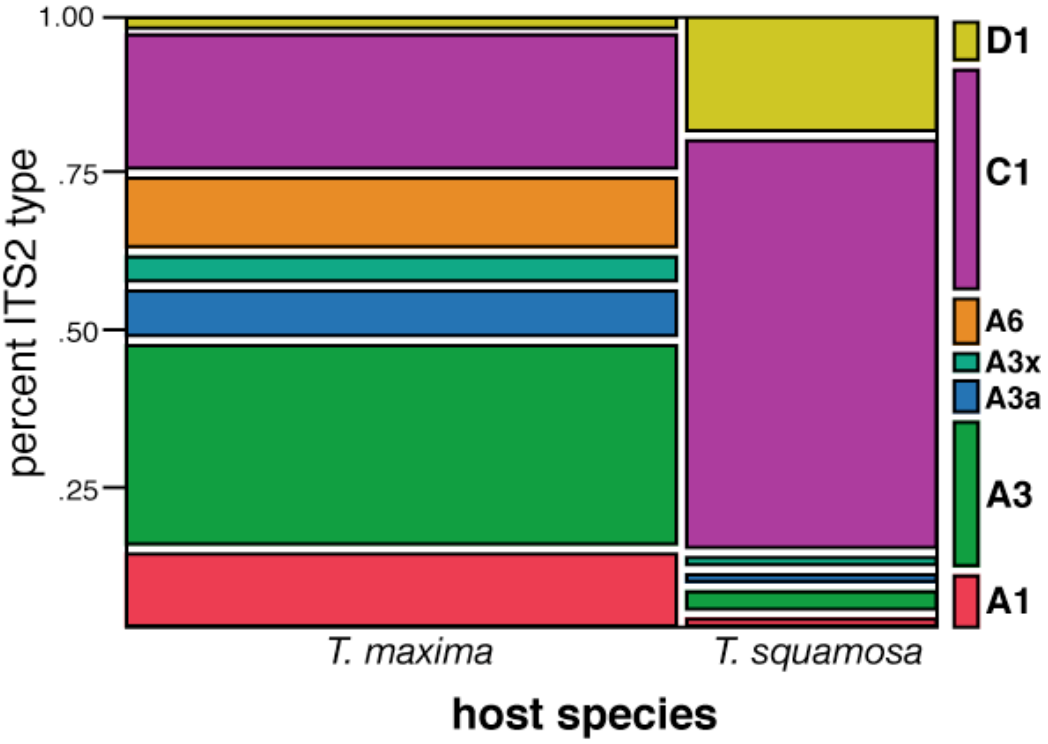


Figure 1.10 Mosaic plot of ITS2 types within *T. maxima* and *T. squamosa*

Symbiont phylotypes were unevenly distributed between the two host species (X^2=76.184; p<.0001). Width of the columns is proportional to number of samples from each host and the Y-axis represents the proportion of each phylotype identified from each host. See Table 1.6.

	A1	A3	A3a	A3x	A6	C1	D1
T. maxima	20	51	13	8	19	35	3
	9.22	23.5	5.99	3.69	8.76	16.13	1.38
T. squamosa	2	3	2	2	0	46	13
	0.92	1.38	0.92	0.92	0	21.2	5.99

Table 1.6
Contingency table used to calculate the Chi squared statistic to test the null hypothesis that the most common symbiont phylotypes were equally partitioned between the two host species. The top row for each species is the number of individuals hosting each type and the bottom row is the percent of the total (X^2=76.184; p<.0001 for 217 host individuals analyzed).

Biogeographic distribution

Symbiont phylotypes were partitioned by geography. For *T. maxima*, ITS2 types A6 and A3x were only found in the Pacific Ocean. Type A3a was found in several *T. maxima* individuals from PNG and the Maldives and a single individual in East Africa but was not observed from other regions in the IWP. Phylotype A1 was the only *Symbiodinium* lineage found in *T. maxima* from the Red Sea and was not documented outside of the Red Sea. ITS2 type A3 was the most common symbiont in *T. maxima* across the IWP. Lineages A6 and A3x varied from A3 by a single base pair, and were hosted by *T. maxima* throughout the Pacific with A3x only occurring as far west as Vanuatu and A6 represented in a few individuals in PNG. A3, A6 and A3x were the only symbionts identified from *T. maxima* in Thailand and from four islands in the Central South Pacific Ocean: Ofu, Rarotonga, Aitutaki and Moorea but were not identified in the Indian Ocean or the Red Sea (Figure 1.11).

The distribution of *Symbiodinium* phylotypes in *T. squamosa* was more specific than *T. maxima,* which hosted more diverse symbionts. Although C1 was identified in approximately 20% of the *T. maxima* individuals primarily from the Western Pacific and East Africa, this lineage was present in 68% of *T. squamosa* individuals distributed across the IWP. There was no site where *T. squamosa* was sampled that it did not host phylotype C1. A3 symbionts were only identified in *T. squamosa* collected in the Maldives and other clade A lineages were found from *T. squamosa* from PNG and a single specimen from Fiji. In all other areas this host species was specific for phylotype C1, even at shallow depths.

ITS2 type D1 was common in *T. squamosa* populations collected in PNG. D1 was also identified in a *T. squamosa* individual as well as two *T. maxima* individuals from the Maldives. Clade D symbionts were not recorded from the Red Sea or the Pacific Ocean east of PNG.

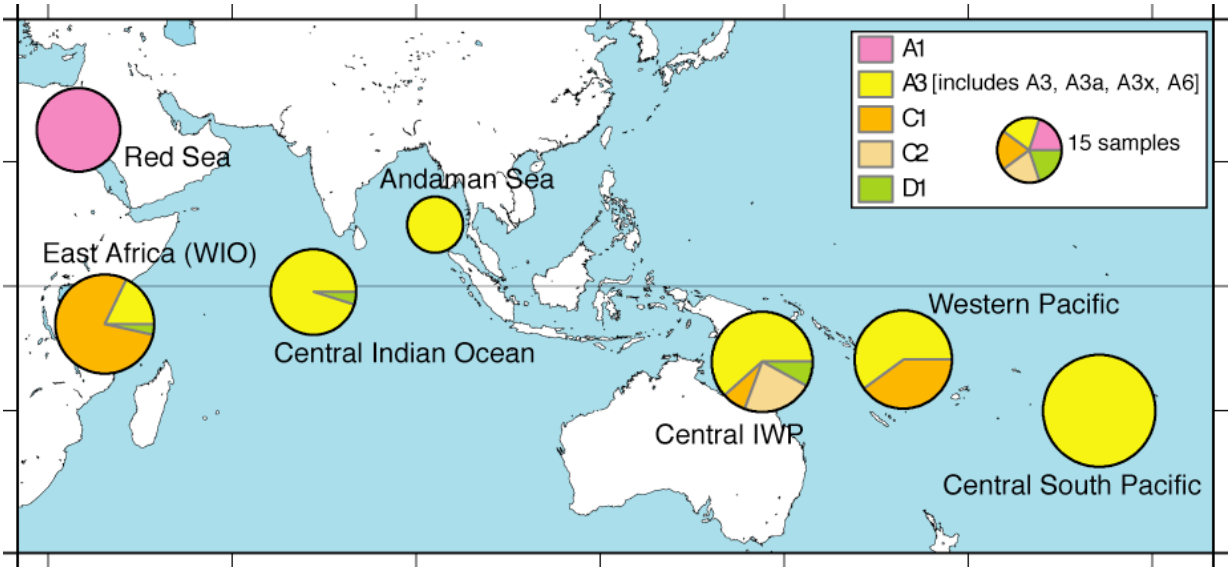


Figure 1.11 Distribution of ITS2 types in *T. maxima*

Yellow included all of the lineages closely related to phylotype A3, which were also colored yellow in the phylogenetic trees (Figures 1.2 through 1.5). The size of the circles was scaled by the number of samples from each region. The single instances of phylotypes A4 in the West Indian Ocean and C66 in the Central IWP were omitted. Note the gradient of declining diversity across the Pacific Ocean.

DISCUSSION

I evaluated three sets of hypotheses to explain the distribution of *Symbiodinium* in giant clams: (1) *Symbionts are organized in tridacnid hosts based on abiotic environmental factors.* I compared the depth distributions and reef environment for each of the common phylotypes using ANOVA and correspondence analysis. (2) *Symbionts are organized within clams based on host specificity.* I tested for certain symbiont types isolated in individual host species. (3) *Symbionts are organized based on biogeography*. The effect of geographic region and distance from the marine center of biodiversity as primary organizing factors on the biogeographic patterns of *Symbiodinium* from giant clams were also investigated with my data.

(1) Abiotic factors controlling the distribution of *Symbiodinium* in *Tridacna*

The results showed that *T. maxima* and *T. squamosa* lived in different habitats so two alternative hypotheses are possible: (1) clams were adapted to certain habitats and therefore acquired the symbionts present in that habitat because that is where the larvae settled or (2) clams acquired certain symbionts and in order to maintain the successful association, they evolved to live in habitats appropriate for those *Symbiodinium*. To differentiate between these hypotheses, I investigated abiotic factors that may have sorted the symbionts into host lineages. The results supported the hypothesis that symbionts were adapted to particular depth ranges and reef environments but that host specificity also partially structured symbiont distribution within the two giant clam species.

Depth range

Each of the common symbiont types in giant clams was partitioned into particular depth distributions for each of the two species. The zonation in phylotypes A3, A3a, A3x, A1, C1 and D1 (Figure 1.7) supported the hypothesis that clams chose depth adapted symbionts appropriate to their habitat. A1, A3a, C1 and D1 were most common at deeper depths for both *T. squamosa* and *T. maxima,* suggesting that perhaps they are better adapted to low light conditions. *T. squamosa* lived approximately 5 m deeper than *T. maxima* (Figure 1.6) and primarily hosted phylotype C1 although this symbiont type was also observed in shallow dwelling hosts. Experimental data showed that *T. squamosa* is a functional heterotroph and depends on filter-feeding to supplement the photosynthate donated by its resident *Symbiodinium* populations (Jantzen et al. 2008). *T. squamosa* primarily hosted ITS2 types C1 and D1 over their depth range and differences in the depth distribution of C1 and D1 were not statistically significant indicating that both phylotypes have similar depth ranges averaging between 10 and 11 meters. However, I did identify ITS2 type C1 symbionts from individuals in less than 2 meters of water and they have been documented in shallow dwelling cnidarians suggesting that they are tolerant of high light conditions (Sampayo et al. 2007; Kuguru et al. 2008). These results suggested that in the *Tridacna* host system, different symbiont lineages have particular functional capabilities and supported the hypothesis that *T. squamosa* associated with depth-adapted symbionts because they were available in the water column when the larvae recruited to deeper reefs. Perhaps deeper dwelling *T. squamosa* preferentially selected these symbiont lineages because they photosynthesize effectively than alternate types under low light conditions and are more efficient at meeting their energetic requirements unfulfilled by filter feeding.

T. maxima was common across a broader range of depths than *T. squamosa* and was especially prevalent on shallow reefs. Although *T. maxima* hosted diverse symbionts, members

of clade A were the most common. Cnidarian hosts living in shallow water also hosted phylotypes from *Symbiodinium* clade A (Toller et al. 2001; LaJeunesse 2002) supporting the hypothesis that most clade A symbionts are high irradiance adapted and thus more common in shallow environments. Phylotype A3a, recently diverged from the common shallow dwelling phylotype A3, appeared to be depth adapted, although closely related types including A3x and A6 are shallow water specialists like the ancestral A3 lineage. ITS2 type A3a was found in deeper dwelling *T. maxima* suggesting that this species which hosted clade A symbionts across its range has evolved recognition mechanisms for symbionts from clade A because they usually settled on shallow reefs.

Many cnidarian symbionts exhibit depth zonation. Corralimorphs and several species of scleractinian corals hosted certain symbiont types in shallow water and different symbionts in the deeper part of their range (Sampayo et al. 2007; Frade et al. 2008; Kuguru et al. 2008). *Tridacna* species were partitioned into different depth profiles and hosted appropriate symbionts within a narrower range of depths. Within the more broadly distributed *T. maxima*, certain phylotypes exhibited specific depth ranges. Within the deeper part of their ranges, *T. maxima* was more likely to host ITS2 types A1, A3a or C1 and *T. squamosa* was more likely to host phylotype C1 or symbionts from clade D but rarely hosted the A3a or A1 lineages.

Clade D is less productive than other *Symbiodinium* lineages but more thermo-tolerant according to several authors who suggested that it may be prevalent as a background population in many hosts (Baker et al. 2004; Fabricius et al. 2004; Rowan 2004; Thornhill et al. 2006; Mieog et al. 2007; Smith 2008; Oliver and Palumbi 2009). Although in some host species, deeper dwelling individuals hosted symbionts from clade D (Kuguru et al. 2008), in other species, shallow dwelling, heat stressed individuals hosted symbionts from clade D (Oliver and Palumbi 2009). In this study, 16 out of 231 clams hosted ITS2 type D1 *Symbiodinium* and individuals hosting D1 symbionts ranged from 1 to 20 meters deep. They did not appear to be limited by high light conditions because three *T. maxima* individuals hosted *Symbiodinium* ITS2 type D1 and were living in less than 2m of water. The average depth for D1 across both host species (9 m +- 5.5 m) was deeper than the other phylotypes but the large variance suggested that they may be common across a variety of depths and that direct sequencing may have underestimated their abundance as background symbionts in giant clams. *Tridacna* at a variety of depths may host small refuges of clade D symbionts but in lower irradiance habitats at deeper depths, these tolerant symbionts dominated the host and can be observed using direct sequencing methods. However in shallow conditions this lineage of symbionts may persist at minimum background levels, and would be detected by sensitive, quantitative techniques such as real time PCR.

Reef Environment

Individual microenvironments vary around an island and across a single reef. On fringing reefs exposed to open ocean, high energy water impacts shallow dwelling organisms at shallow depths because waves from the open ocean crash into the reef structure. However organisms living on the forereef or on fringing reefs have easy access to new symbionts and the circulation patterns help disperse larvae as well as symbionts. Below the turbulent, high-energy zone calm deeper depths are constantly flushed by clear, cool water. Behind fringing reefs, in lagoons, or on patch reefs, water circulation is restricted, temperatures are higher and dissolved gas content is lower. Organisms are partitioned into microenvironments depending on their

tolerance for wave energy, circulation and degree of exposure to potential symbionts through delivery and dispersal among other factors.

I evaluated the partitioning of symbiont types into lagoon environments, patch reefs and fringing reefs. In ordination space visualized by correspondence analysis, A3 and A6 were closely associated with lagoon environments implying that these phylotypes were adapted to shallow depths and could withstand limited circulation and exposure to open ocean water (Figure 1.9). This was particularly evident in Moorea and the Cook Islands where I exclusively sampled *T. maxima* from restricted, shallow lagoon environments and they exclusively hosted phylotype A3 symbionts and closely related lineages such as A6. Other ecological evidence suggested that symbionts from clade A were common in shallow lagoon environments (Toller et al. 2001; LaJeunesse 2002). Barrier reefs form around young Pacific islands and create shallow lagoon environments. Because these islands do not experience extensive continental run off, the water is also clearer. These symbionts may be adapted to the high irradiance conditions associated with clear, shallow water.

ITS2 types A1, A3a, C1 and D1 were more common on patch reefs and fringing reefs. Clade D symbionts were closely associated with fringing reefs for *Tridacna* but clade D *Symbiodinium* from corals were common in both shallow lagoons and deeper fringing reefs in American Samoa (Oliver and Palumbi 2009). My results were complicated by the sampling scheme inherent in this observational study because not all variables were independent and I could not design a fully crossed sampling scheme. Samples collected on fringing reefs, like many of those hosting clade D symbionts, experienced increased water movement and access to dispersing currents. However fringing reef habitat was not independent from the effects of deeper depths. In Vanuatu, Fiji and Zanzibar, I sampled *T. maxima* and *T. squamosa* exclusively from fringing reef and patch reef habitats. Phylotype C1 symbionts predominantly associated with these reef environments although several clams hosted clade A as well. Exceptions to this pattern included Sri Lanka where clams collected from fringing reefs hosted phylotype A3 and Kenya where clams from restricted lagoon environments hosted ITS2 type C1. In addition, although clade A was mostly associated with shallow water, fringing reefs bordered active margins in the Red Sea and there were no shallow lagoons because it is such a young system. Therefore, ITS2 type A1, specific to the Red Sea in giant clams, was closely associated with fringing reefs in ordination space. Consistent with other regions, most *T. squamosa* from the Red Sea hosted phylotype C1, a type common on both fringing reefs and patch reefs.

Currents regularly deliver new potential partners to hosts (Howells et al. 2009). The symbiont community was more diverse in *T. maxima* and *T. squamosa* living on fringing reefs or forereefs and could be explained by increased availability of new, potential symbionts because currents deliver dispersing symbionts more regularly to the forereef than the more restricted lagoon. Temperatures are also more constant because of wave action and currents that facilitate mixing over the forereef whereas the lagoon warms and cools with the tidal cycle. According to this hypothesis, because *T. squamosa* consistently live on fringing reefs, I would expect them to host more diverse symbiont populations. However, these results showed that *T. maxima* hosted more diverse symbionts regardless of reef environment. The data supported the hypothesis that symbiont phylotype in giant clams was partially ordered by habitat but that some hosts are specific for certain symbionts, regardless of habitat.

(2) Host specificity for ITS2 types

T. maxima and *T. squamosa* are sister taxa and the most widespread of the giant clam species (Schneider 2002). Many lineages of *Symbiodinum* also exhibit cosmopolitan distributions. Hundreds of *Symbiodinium* ITS2 types are present in diverse host taxa and reef environments across the IWP (for compilation and analysis of ITS2 types see: Correa and Baker 2009 of the many) but *T. maxima* and *T. squamosa* consistently hosted only a few phylotypes. The two most common *Symbiodinium* phylotypes identified from these giant clams were ITS2 types A3 and C1 and none of the lineages identified from *Tridacna* were specific to giant clams; most are also common symbionts in a diverse group of host organisms. In many organisms clusters of closely related divergent types have evolved from these ancestral lineages of *Symbiodinium* and/or intragenomic variation confound efforts to identify the dominant symbiont population so higher resolution markers or other techniques may be necessary to identify mixed populations (Thornhill et al. 2007; Correa and Baker 2009; Fay et al. 2009; Sampayo et al. 2009). However, as a derived metazoan, symbiont diversity in giant clams appears to be limited and easy to investigate using direct sequencing methods. Symbiont populations in *Tridacna* were less variable and more specific because they reproduce sexually at each generation. In contrast, other hosts, such as corals or forams frequently reproduce asexually and can indefinitely propagate isolated (and potentially evolving) symbiont populations within a single host lineage via fragmentation or budding. Under these conditions the symbiont lineage could diversify within the host lineage or coevolve as a result of strict, reciprocal adaptation processes. Each generation of clams reacquires a new population of ancestral symbiont types from the water column after sexual reproduction; therefore, a particular lineage of *Symbiodinium* has no opportunity to adapt or diversify, isolated from the free-living dinoflagellate community, within a single lineage of clams.

Although both A3 and C1 were present in both giant clam species, they were not uniformly distributed, indicating some degree of host specificity. Contingency analysis identified patterns in the distribution of symbionts and showed that the six most common ITS2 types were unequally partitioned within the two host species (Figure 1.10), thus rejecting the null model that dinoflagellate symbionts are randomly distributed in giant clam hosts and all symbiont types are pandemic (for microbial examples see: Martiny et al. 2006). My data supported the alternative hypothesis that modern *Tridacna* species are specific for particular lineages of *Symbiodinium*. *T. maxima* primarily hosted symbionts from the A3 lineage and *T. squamosa* primarily hosted symbionts from the C1 lineage but a geographic mosaic of selection pressure selected for alternative symbiont lineages under certain conditions in certain regions.

(3) The Biogeography of *Symbiodinium* in *Tridacna*

Areas of highest biodiversity for the giant clam symbionts occurred in boundary regions such as Papua New Guinea between the Pacific and Indian Oceans and along the east coast of Africa between the Indian Ocean and the Red Sea. Rare *Symbiodinium* types found in only a few specimens were identified from these regions but across the rest of the giant clam distribution, symbiont diversity was limited to six phylotypes. A single specimen each of phylotype C66 and phylotype A4 were identified in the *T. maxima* population from Kenya. Two *T. maxima* individuals hosted ITS2 type C2 in PNG. However the majority of clams from both sites hosted symbionts from the other more common phylotypes. Limited connectivity between marine organisms despite planktonic larval phases (Paulay and Meyer 2006; DeBoer et al. 2008; Kochzius and Nuryanto 2008; Reaka et al. 2008) indicated that barriers to dispersal exist at

boundary regions, limiting diversity to narrow zones. Fine scale sampling within these regions would explore community diversity in giant clam symbionts at the geologic boundary between two major ocean basins.

Intermediate levels of symbiont diversity were observed in clams from several localities, such as Fiji, New Caledonia and the Maldives. *T. squamosa* consistently hosted ITS2 type C1 across the IWP but other phylotypes were region specific. Although it has been reported in other taxa from the Pacific (Smith 2008), in giant clams, clade D was not observed farther east than Australia and only twice in the Indian Ocean suggesting that it is rare in *Tridacna* outside of the Central IWP. Phylotype A1, also known from other taxa (LaJeunesse et al. 2009), was only identified in clams from the Red Sea and was the only symbiont in *T. maxima* from the Red Sea, suggesting that under conditions unique to the region, A1 symbionts outcompeted the phylotypes that inhabited *T. maxima* over the rest of its distribution (Chapter 2). Phylotype A3 appeared to be a basal generalist that has diversified through time to yield derived lineages specific to particular geographic regions of the giant clam distribution (Figure 1.12). ITS2 type A3a lived in deeper habitats and was only documented from the Indian Ocean. A3x and A6 were only found in the Pacific Ocean, often in shallow lagoon environments suggesting that this lineage diversified in the remote Pacific to take advantage of certain habitat conditions. Each of these phylotypes was a single step from the ancestral A3 phylotype at the ITS2 locus. Evidence of diverging lineages that specialize to perform in particular depth niches has been shown for vertically transmitted symbionts in corals (Sampayo et al. 2007). However, clams derive their symbionts exclusively from the environment and have limited opportunity to coevolve with populations of symbionts. These observations were consistent with the hypothesis that under a mosaic of selection, symbiont lineages evolve specificity within limited geographic regions. If closely related symbiont types are geographically and ecologically structured across the marine environment, hosts may evolve specificity for certain types based on their own niches.

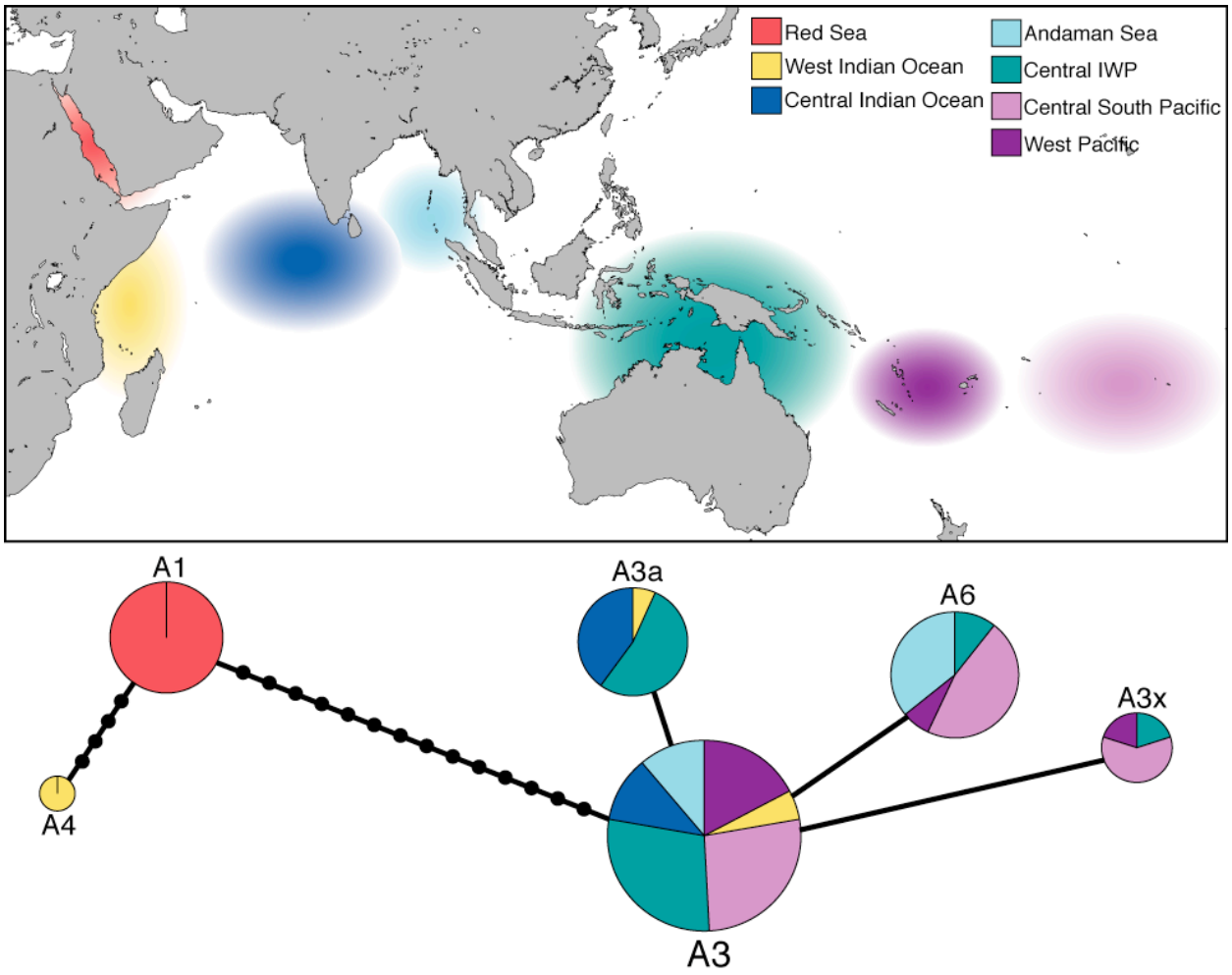


Figure 1.12 Network diagram for clade A *Symbiodinium* in *Tridacna*

Pie slices represented the proportion of each phylotype within clade A identified from both species in each region. Each black line represented a single nucleotide change within the ~300 base pairs of the ITS2 locus and each black spot represented a hypothetical intermediate genotype. The size of the circles was scaled to sample size; the ancestral A3 type was identified in 63 clams. Notice that ITS2 type A1 was only found in the Red Sea and that ITS2 type A4 was only found in the WIO. Most of the clams from the WIO hosted clade C symbionts and therefore, were not represented in this figure.

The Pacific Ocean

Global marine biodiversity peaks in the Central IWP between Indonesia, the Philippines and Papua New Guinea. Over 15 hypotheses propose mechanisms to explain biodiversity patterns in this region. However, models based on potential processes do not always successfully predict these patterns and I am still gathering the data required to understand the origin of diversity in multiple marine phyla (Rosen 1988; Reaka et al. 2008; Renema et al. 2008). Like many other IWP taxa, *Symbiodinium* diversity in giant clams declined from west to east across the South Pacific. Diversity was lowest around islands in the Central South Pacific, intermediate around Fiji and Vanuatu and highest in the Central IWP.

Representing the center of marine biodiversity, I sampled clams from Northeastern Australia and two different sites in PNG. I found more lineages of *Symbiodinium* in each host species from these localities than anywhere else within the sampling scheme. *T. maxima* hosted A3, A3a, A6, C1, C2 and D1. *T. squamosa* hosted A3, A3a, A3x, C1 and D1. This diversity pattern suggested two alternative hypotheses: (1) *Symbiodinium* from more diverse lineages are available to affect larvae in the Central IWP and (2) *T. maxima* and *T. squamosa* are generalists in the Central IWP and host whatever symbiont first infected the larva. I rejected the first hypothesis because other hosts from remote locations also hosted diverse lineages of *Symbiodinium* (Pochon et al. 2007) implying that overall diversity did not limit potential host-symbiont associations outside of the Central IWP. The specificity hypothesis was tentatively supported because these symbionts only participated in associations with giant clams from diverse regions. However with these data I could not distinguish by which mechanisms specificity relaxed outside this region. Additional sampling and experiments designed to infect generalist clam larvae derived from the Central IWP compared to more specific clams from regions with limited holobiont diversity would further address these ideas.

T. maxima and *T. squamosa* differed in their degree of specificity in the intermediate zone, which included various localities in Fiji, Vanuatu and New Caledonia. At intermediate longitudes, *T. maxima* hosted diverse symbionts including: A3, A3x, A6 and C1 symbionts but *T. squamosa* populations from this region hosted exclusively C1 with a single exception from Fiji. These data suggested that *T. squamosa* specificity for ITS2 type C1 increased immediately east of the Central IWP. In contrast, *T. maxima* hosted multiple alternative symbiont phylotypes throughout the Western Pacific indicating that it is a generalist host across a larger portion of its distribution, and its potential for broad association wasn't limited until the break between Fiji and Samoa.

Nearly identical *Symbiodinium* sequences were isolated from all *T. maxima* from Samoa, the Cook Islands and French Polynesia in the Central South Pacific. *T. maxima* from reefs around the isolated islands of Ofu, Rarotonga, Aitutaki and Moorea exclusively hosted ITS2 type A3 *Symbiodinium* and closely related lineages such as A3x and A6 (Figure 1.11). Alternative hosts from French Polynesia and Samoa did host alternative phylotypes from clades C and D (Magalon et al. 2006; Smith 2008). Giant clam larvae would have access to these phylotypes since they acquire symbionts from the water column as larvae, and I did observe them in clams from other geographic regions. However, with these data, I showed that in the eastern part of their distribution, *T. maxima* were specialized for phylotype A3 and its closely related derivative lineages (Figure 1.12). I was unable to sample *T. squamosa* because they had been overfished to near extinction around these islands and as a result, I may have missed additional symbiont diversity sequestered in rare hosts. However, *T. squamosa* from Fiji and New Caledonia exclusively hosted C1. The longitudinal gradient observed via these data did not suggest that

specificity would relax again; therefore, I expect that rare, refuge populations of *T. squamosa* in Samoa and the Cook Islands would also be specific for ITS2 type C1.

Symbiodinium diversity in *Tridacna* declined across the South Pacific Ocean. *Symbiodinium* populations in other host taxa also supported this observation of increased diversity in the Central IWP and limited diversity isolated islands of the Pacific (Van Oppen et al. 2005; Goulet et al. 2008) although in a few, isolated Pacific localities that were well sampled, diverse symbionts associated with alternative hosts (Pochon et al. 2007; Stat et al. 2009). I did not record endemic lineages of *Symbiodinium* specific to giant clams from the remote Central South Pacific and if considered representative, these observations would reject the museum of diversity hypotheses. These arguments propose that the Central IWP is the most diverse because endemics evolve in allopatry and accumulate in the center where they are less likely to go extinct. Patterns in *Symbiodinium* diversity across the Pacific supported the cradle of diversity hypotheses, which attribute high diversity in the Central IWP to a complex geological history, local ecological variability and/or inherent diversity of the region and which implies that symbiont diversity originated in the coral triangle and dispersed to isolated reefs (of the many: Pianka 1966; Rosen 1988; Emerson and Kolm 2005; Marshall 2006; Briggs 2007).

The Indian Ocean

Approximately 8,000 km from the center of biodiversity, the West Indian Ocean (WIO) is depauperate compared to the rest of the IWP system. The Indian Ocean is the most under-sampled of the world's major oceans and much of the sampling has been concentrated in the WIO along the East African coast. Of the studies that examined *Symbiodinium* diversity in this region, most used relatively low resolution markers, such as LSU, which obscured finer scale diversity (Burnett 2002; Baker et al. 2004; Visram and Douglas 2006; Macdonald et al. 2008; Sebastian et al. 2009). Even so, these studies almost exclusively described symbionts from clades C and D and only one study identified a single sequence from clade A (Visram and Douglas 2006).

I confirmed that clade C, specifically ITS2 type C1, was the most common symbiont in giant clams as well as corals along the East African coast. *T. squamosa* exclusively hosted C1 and it was identified in 76 % of *T. maxima* individuals from three localities although this symbiont was a minority symbiont for other localities across the IWP. Among diverse *Symbiodinium* lineages associated with the remaining 24 % of *T. maxima* and, in addition to common phylotypes, I identified two rare ITS2 types, C66 and A4, not identified from *Tridacna* elsewhere in the IWP. Although novel types were in low abundance, the diversity of symbionts hosted by *T. maxima* from this region approached the high diversity hosted by *Tridacna* in the Central IWP and indicated that *Symbiodinium* exhibit a secondary center of diversity in the WIO.

In the Central Indian Ocean I sampled giant clams from multiple sites in the Maldives, a single site in Sri Lanka and two sites in Thailand. The Maldives exhibited intermediate levels of diversity but *T. maxima* from Sri Lanka were specific for phylotype A3 and clams from Thailand were specific for A6, a closely related lineage, otherwise known only from the Pacific. Samples from the Andaman Sea were collected from fringing reefs along a continental margin, analogous to reefs I sampled in the WIO. In contrast to isolated island systems, organisms along continental coastlines more easily disperse between localities along continuous reef structure and potentially contribute to more diverse communities. Diversity on islands is proportional to the size of island habitat and the distance from the source population (MacArthur and Wilson 1967), neither of which should limit diversity in continuous continental reef systems. However, while

the WIO communities that I sampled from fringing reefs in Kenya and Zanzibar were quite diverse, many of the Western Pacific reefs that I sampled exhibited greater diversity despite being islands of volcanic origin, located thousands of kilometers from other reefs and without obvious sources of dispersing immigrants.

Surprising symbiont diversity patterns in *T. maxima* samples in the Andaman Sea suggested two working hypotheses: (1) Clams from islands in the middle of the Indian Ocean are more diverse than those from coastal areas bordering the Andaman Sea because they are less specific. Or (2) the *Symbiodinium* diversity gradient in the Indian Ocean also runs from West to East, decreasing closer to the Central IWP, in opposition to the longitudinal gradient in the Pacific, which decreases away from the Central IWP. Possibly coastlines around the Andaman Sea are depauperate as a result of abiotic factors, for example, sediment from river deltas draining onto reefs or destructive human development. However, *Symbiodinium* phylotypes from clade C and clade D were isolated from samples of a single coral species from Thailand (Burnett 2002; Lien et al. 2007) and this indicates that giant clams from this region were specific for clade A even though new generations of larvae had access to alternate symbiont types. Thus limited evidence supported the hypothesis that clams from this region were more specific than elsewhere in their range. The diversity of *Symbiodinium* in alternative hosts from Sri Lanka is unknown and this hypothesis cannot be further tested now.

The second idea was interesting because Thailand is remarkably close to the global center of marine biodiversity in the Central IWP but my data indicated that across the Indian Ocean, symbiont diversity in giant clams was lowest at this locality. Only a single phylotype, ITS2 type A6, was observed from *T. maxima* collected on the Indian Ocean side of the Southeast Asian peninsula. This symbiont was common in the Central South Pacific and documented from the Central IWP but it was not observed elsewhere in the Indian Ocean. *T. squamosa* samples from the Andaman Sea were not available but perhaps rare individuals that I was unable to sample, hosted additional symbiont diversity. The global biodiversity gradient extends out both east and west from the Central IWP for other marine organisms and studies of other taxa showed a biogeographic diversity break between the coral triangle and the East Indian Ocean (Hoeksema 2007; Bellwood and Meyer 2009). However, very few *Symbiodinium* hosts have been sampled from this region and until community diversity across alterative hosts can be documented, I cannot convincingly address a hypothesis about a reverse gradient across the Indian Ocean for dinoflagellate symbionts.

The Red Sea

Tridacna maxima from the Red Sea, was specific for ITS2 type A1 *Symbiodinium* (Chapter 2). Two *T. squamosa* from the Red Sea also hosted A1 but most individuals hosted ITS2 type C1. ITS2 type A1 is not recorded from *Tridacna* anywhere else in this study or in the literature. The Red Sea rifted open relatively recently, within the last 5 million years ago, and has since been isolated by low sea level stands several times in the last 120,000 years. A1 in giant clams was determined to be an endemic holobiont in early stages of succession in the Red Sea. The A1 lineage is an infectious, widespread generalist that can tolerate high light conditions in this newly formed ocean. *T. squamosa* appears to have reverted to its "normal" symbiont, ITS2 type C1, which dominated in *T. squamosa* across the IWP. *T. maxima* was slower to evolve beyond the early successional stage and as an intermediate holobiont, it maintains the infectious ITS2 type A1 (Chapter 2). I speculate that as these reefs mature on a geological time scale, *Symbiodinium* genetic diversity will increase and *Tridacna* from the Red

Sea will acquire symbionts that are common in alternative hosts and neighboring giant clam populations such as those along the East African coast in the WIO.

Implications for the evolution of symbiosis in *Tridacna*

The giant clam lineage evolved over the last 50 million years and the genus *Tridacna* first appeared in the fossil record in the Miocene. Scleractinian corals have hosted symbionts since the Triassic and soritid foraminifera, which also host dinoflagellates, diversified in the Cretaceous (Stasek 1962; Loeblich Jr. and Tappan 1988; Stanley and Swart 1995; Richardson 2001; Harzhauser et al. 2008). In corals and forams, dinoflagellate endosymbionts are housed intercellularly but tridacnids host symbionts in tertiary tubules that originate in the digestive system (Trench et al. 1981; Norton et al. 1992). Giant clams and other bivalves that host *Symbiodinium* (Farmer et al. 2001) are derived crown group metazoans and only reproduce sexually. They reacquire their symbionts at each generation and as a result they host a limited number of basal phylotypes. The fossil record, the incomplete incorporation of symbionts into host cells and the limited symbiont diversity shown here together suggested that photosymbiosis evolved relatively recently in mollusks and that they may represent an intermediate step in the evolutionary trajectory towards an obligate, fully integrated, dinoflagellate photosymbiosis. Their tendency to host symbionts in their digestive system also suggested that the association between mollusk hosts and *Symbiodinium* may still be relatively labile.

Clams in the Central South Pacific Ocean were farthest from the center of diversity and exclusively hosted three closely related phylotypes, A3, A3x and A6, which only differed by a single base pair within the ITS2 locus (Figure 1.13). Clade A is the most basal lineage within the genus *Symbiodinium* and mostly closely related to free-living ancestors (Pochon et al. 2006; Stat et al. 2008). Experimental data suggested that Clade A is less cooperative than other more derived clades (Stat et al. 2008). Representatives from clade A are also the easiest to culture in the lab indicating that they easily survive outside their host compared with members of clade C, many of which cannot be easily cultured (Rowan 1998; Santos et al. 2001; Ishikura et al. 2004). These lines of evidence supported the hypothesis that phylotype A3 and its derivative lineages were prevalent in the Central South Pacific because they are less dependent on a host and can more easily disperse to isolated islands. *T. maxima* in the Red Sea, another isolated environment, exclusively hosted ITS2 type A1. Clams colonized the reflooded Red Sea from the WIO in the last 12,000 years and data indicated that they replaced their original symbionts with lineage A1 in the Red Sea (Chapter 2). The basal lineage from clade A was more likely to independently disperse into the Red Sea as a free-living organism and/or survive outside a host under challenging abiotic conditions.

T. squamosa and *T. maxima* are closely related and shared a common ancestor in the mid Miocene. Both fossil and molecular evidence showed that *T. maxima* diverged from the common ancestor several million years before *T. squamosa* (Schneider and Foighil 1999; Harzhauser et al. 2008). *T. squamosa*, the younger lineage, evolved to exploit a deep water niche and reverted to the ancestral mollusk lifestyle of filter feeding to meet its energetic requirements. *T. squamosa* was a functional heterotroph and did not depend on photosymbiosis like *T. maxima,* a functional autotroph (Jantzen et al. 2008). *T. squamosa* was specific for the symbiont lineage, ITS2 type C1. Both its derived, phylogenetic position and its inability to live in culture suggested that clade C symbionts are better, more cooperative partners. Clade C is less costly to the host for two reasons: (1) it donated more photosynthate to hosts (Stat et al. 2008) and (2) it photosynthesizes effectively in deeper water. Therefore, a C1 host would be released from the

energetic concessions required to support mollusk life in a high irradiance zone, including tolerance of increased oxidative stress and production of sunscreen pigments (Muller-Parker and D'Elia 1997; Yakovleva et al. 2009). If a recently diverged host has adapted to exploit a deep water niche where it is released from the pressure to tolerate high light conditions because it is not energetically dependent on symbiosis, the host may only accept association with the most cooperative symbionts. Perhaps specificity for ITS2 type C1 was the freedom to reject the higher costs associated with hosting symbionts from clade A. *T. squamosa* only occasionally hosted ITS2 type A1 in the Red Sea where a recent dispersal event to a region with harsh conditions forced *T. maxima* to exclusively host A1 symbionts (Chapter 2) although in all other regions, *T. maxima* was less specific than *T. squamosa*. *T. maxima* may not actually be less specific than *T. squamosa* but rather a more obligate partner. *T. maxima* tolerated less cooperative symbionts from clade A throughout its distribution because it is derived from a lineage of functional autotrophs that already incurred the high costs associated with supporting a symbiont population in high light conditions. The specificity of *T. squamosa* suggested that it has reverted to an ancestral state of mixotrophy, which allowed the host to maximize its benefits and minimize its costs by selecting for associations with only the most cooperative symbiont lineages.

CONCLUSIONS

Across their IWP distribution, *T. maxima* and *T. squamosa* consistently hosted two common symbiont lineages, *Symbiodinium* ITS2 types A3 and C1. *T. maxima* hosted more diverse symbionts and was less habitat restrained than *T. squamosa*, but phylotype A3 was a common symbiont in most regions. *T. squamosa,* which lived in deeper forereef environments, was specific for *Symbiodinium* ITS2 type C1. Although a few individuals hosted other symbiont phylotypes in PNG and the Maldives, outside of diverse regions, *T. squamosa* only hosted phylotype C1. These data indicated that the most common members of the clam-dwelling *Symbiodinium* population are widely distributed across the IWP and support the 'everything is everywhere' hypothesis borrowed from microbial biogeography (Martiny et al. 2006). However, the common ancestral phylotypes were partitioned by host species and the less common symbiont types were sampled only from restricted ranges supporting an alternative hypothesis that the evolution of association between *Symbiodinium* and *Tridacna* exhibited regionally variable biogeographic patterns. The more recent divergence of *T. squamosa* and its adaptation to deeper environments allowed it to selectively accept only the most cooperative symbionts because this host species was no longer dependent on symbiosis to meet its energetic requirements. The distribution of *Symbiodinium* in *Tridacna* supported the hypothesis that evolutionary history of a host lineage and variable abiotic factors contributed to mosaic selection regimes across the Indo West Pacific.

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CHAPTER 2

Evolution of the symbiosis between the dinoflagellate, *Symbiodinium*, and the giant clam, *Tridacna maxima*, in the Red Sea

ABSTRACT

The Red Sea is an extension of the Indo West Pacific (IWP) biogeographic province. Red Sea coral reefs survive at high latitudes and tolerate extreme salinity and seasonal temperatures. Narrow straits in the south connect the Red Sea to the rest of the IWP but circulation is limited. Despite these challenges, diverse communities have evolved including a variety of organisms that host endosymbionts from the dinoflagellate genus *Symbiodinium*. *Tridacna maxima* collected along the Egyptian coast of the Red Sea contained only one unique lineage of *Symbiodinium*. Giant clam mantle tissue was collected from four sites across 350 km of the Red Sea coastline at a variety of depths. When the symbiont rRNA was sequenced and analyzed, all 20 samples were closely related to *Symbiodinium* ITS2 type A1. This symbiont lineage was previously documented in jellyfish and corals from the Red Sea and other oceans but never in giant clams. Although other symbionts exist in alternative Red Sea hosts, *T. maxima* was specific for the A1 linage of *Symbiodinium*. This successful but previously unreported symbiosis most likely evolved after independent colonization events by host and symbiont since the last glacial maximum, about 12,000 years ago, when sea level dropped and isolated the Red Sea from other oceans. This endemic holobiont may represent evolutionary flexibility as the symbiosis adapts to a newly developed ocean system after the reflooding of the Red Sea.

INTRODUCTION

Red Sea: oceanography, environment and history

The Red Sea is small and isolated. It is 2200 kilometers long and only 300 km wide at the widest point with a narrow, single, natural opening at its southern end. The rift ranges between 600 and 1000 meters deep and splits into two bays in the north. The deepest and most active part of it follows the Gulf of Aqaba to the northeast. The Gulf of Suez to the northwest is much wider and shallower. Neither have a natural outlet; the Suez Canal was cut in 1869. In the south, between Yemen and Ethiopia, the Red Sea is connected to the Western Indian Ocean (WIO). The straits at Bab el Mandab opened and the rifting basin flooded approximately 5 million years ago (mya) (Coleman 1993). The modern opening is 18 kilometers wide and 130 meters deep (Hassan et al. 2002) and provides the only potential entry to the Red Sea for marine immigrants originating in the tropical Indo West Pacific (IWP) system (Figure 1).

The Northern Red Sea reefs are among the highest latitude reefs in the world, ranging from 25 to 30 degrees north. They experience some of the highest and the lowest measured temperatures that support coral reefs, 32°C in the summer and 22°C in the winter. Freshwater input is absent and the surrounding land is a hot, cloudless desert. Evaporation exceeds sea-water exchange through Bab el Mandab and the salinities reach 40 $^{o}/_{oo}$ in the summer (Hassan et al. 2002). These extreme abiotic conditions likely exert strong selective pressure on organisms living in this isolated ocean system.

The Red Sea: Endemic lineages

Red Sea reefs have evolved in relative isolation because of limited mixing of populations through the narrow straits but are some of the most diverse ecosystems west of Australia (Sheppard 1987; Hughes et al. 2002; Roberts et al. 2002). Phylogenetic analyses indicate that many modern Red Sea endemic lineages have sister groups in the WIO (Meyer 2003; Borsa et al. 2007; Frey and Vermeij 2008). A recently described species of giant clam, *Tridacna costata*, is endemic to the Red Sea and most closely related to *Tridacna maxima* (Richter et al. 2008).

Founding populations of corals, giant clams and other, later immigrants arrived in the Red Sea as larvae on currents that flowed through the straits at Bab el Mandab, but circulation is contingent on climatic conditions. At earlier geologic times with lower sea level stands, Bab el Mandab was virtually closed, restricting flow between the Red Sea and the Indian Ocean (Siddall et al. 2004). Multiple cycles of Pleistocene glaciation have caused sea level to fluctuate over the last five million years since the first appearance of organisms from the WIO (Coleman 1993; Hemleben et al. 1996; Taviani 1998). The modern community is composed of immigrants that entered and colonized Red Sea reefs after Bab el Mandab reopened as sea level rose following the last glacial maximum (LGM), 12,000 years ago (Siddall et al. 2003; Fernandes et al. 2006).

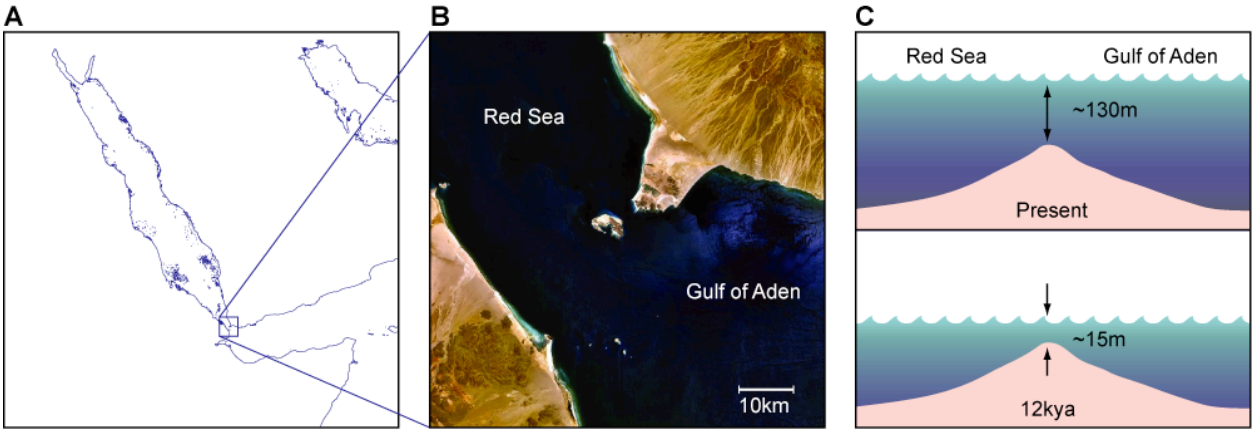

Figure 2.1 The Red Sea, the straits at Bab el Mandab and the Hannish Sill

2.1a The Red Sea is a flooded rift valley between the African plate and the Arabian plate.

2.1b An aerial view (NASA) of the straights at Bab el Mandab where the entrance to the Gulf of Aden and the Indian Ocean is only 18 km wide at the widest point.

2.1c The Hannish Sill, located 120 km north of Bab el Mandab, is only 130 m deep today (top panel). When sea level was lower 12,000 years ago, only 10-15 m of water flowed over this sill, virtually stopping circulation between the Red Sea and the Indian Ocean (bottom panel).

***Tridacna maxima, Symbiodinium* and the holobiont**

T. maxima is abundant in the Red Sea and in the WIO, and its range spans the IWP. The earliest tridacnid ancestors evolved 55 mya in the Tethys Sea, a shallow, warm ocean that formed as Gondwana separated from Laurasia (Rosewater 1965). The Tethyan taxa that lived in the Mediterranean Sea went extinct at the end of the Paleogene but intermediate lineages defined by fossils from reef terraces in Oman indicated that the family dispersed through Arabia and into the Indian Ocean in the mid Miocene (Harzhauser et al. 2008). Today *T. maxima* is the most widespread and the most common giant clam species in the Red Sea and across the IWP (Nuryanto and Kochzius 2009).

Tridacnid clams, their ancestors and other reef building organisms thrive in nutrient poor, tropical waters because they host *Symbiodinium,* a diverse clade of symbiotic dinoflagellates that structurally and energetically support coral reef communities. The symbionts produce an excess of photosynthate and donate it to their host in return for access to phosphorous, nitrogen and a safe living environment (Muscatine and Porter 1977; Muscatine et al. 1981; Klumpp et al. 1992; Hawkins and Klumpp 1995). The extra photosynthate provided by *Symbiodinium* allows giant clams to grow larger than any other living bivalves (Rosewater 1965).

At least eight subgeneric lineages of the endosymbiotic dinoflagellate *Symbiodinium* have been designated by lettered clades and numbered subclades (Coffroth and Santos 2005). For giant clams and other hosts with horizontal transmission, each generation of larvae acquire symbionts from the water column. The definition of specificity used here is the degree to which host and symbiont consistently form partnerships in cases of horizontal transmission. Specificity varies within and between host lineages and geographic ranges. However, for the majority of symbiont specific hosts, environmental conditions appear to mediate preexisting host specificity. Hosts are specific for a certain *Symbiodinium* phylotype within a restricted geographic area and a particular abiotic habitat (LaJeunesse et al. 2004). However, upon exposure to different environmental conditions, some hosts acquire a novel symbiont type or increase the density of a previous background variant (Thornhill et al. 2006; Kuguru et al. 2008; Baskett et al. 2009). As more *Symbiodinium*-hosting organisms are collected from across their entire modern distributions, their symbionts will be cataloged on multiple spatial and temporal scales and symbiont specificity can be assessed for individual host lineages (for examples, see Goulet et al. 2008; Howells et al. 2009).

In this study, symbionts associated with *T. maxima* from the Red Sea were consistently and exclusively shown to be *Symbiodinium* ITS2 type A1. I define an endemic holobiont as a unique pairing of host and symbiont that is unknown from other regions within the distribution of both organisms. This study tested a series of hypotheses about the origins of each partner and the establishment/evolution of this endemic holobiont with respect to the geologic evolution of the Red Sea.

MATERIALS AND METHODS

In 2007, five *T. maxima* individuals were sampled from each of four sites along 350 km of the Egyptian coast of the Red Sea (Figure 2): Dahab (N28.57203 E34.53698), Ras Nasrani (N27.96463 E34.41578), Hurghada (N27.26891 E33.8942) and El Qeseir (N25.87183 E34.41878). At each site, the samples were collected from the same reef, within a radius < 15 meters, and between 3 and 15 meters deep. SCUBA divers used a dulled knife as a wedge to separate the two valves and used a hemostat and small scissors to clip approximately 0.25 grams of tissue from the surface of the mantle. At the surface, the tissue was transferred to tubes containing 80% ethanol and stored at negative 20°C as soon as possible after collection.

In the laboratory, genomic DNA from the Egyptian samples as well as four other sites other sites across the Indo-Pacific (Zanzibar, Australia, American Samoa and Moorea, French Polynesia) was extracted from a small portion of the mantle tissue sample using Qiagen extraction kits. The ITS1-5.8S-ITS2 rRNA locus was amplified using primers S_DINO and L_O developed by Pochon et al. (2001), AmpliTaq Gold (Applied Biosystems) and an MJ PTC-200 thermocycler under the following conditions: 94C for 5 min, 40X(94C for 45s, 58C for 45s, 72C for 2 min), 72C for 5 min. The PCR product was quantitated and purified with ExoSAP-IT (USB/Affymetrix) before being sequenced at the UC Berkeley Sequencing Facility using the primer S_DINO.

The electropharograms were reviewed and trimmed in Geneious and blasted against the GenBank database. Approximately 850 base pairs from the 20 Red Sea sequences in this study aligned with *Symbiodinium* sequences in the GenBank database. The 20 sequences from the Red Sea and five sequences collected from the Indo-Pacific were aligned with reference sequences (Table 2.1 and 2.2) downloaded from GenBank, using MUSCLE (Edgar 2004). The 25 sequences were trimmed to approximately 650 nucleotide positions and realigned using MUSCLE. Further editing of the alignment was not necessary. Phylogenetic hypotheses were inferred using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The GTR+I+invgamma model for sequence evolution was chosen and the analysis was run for 5,000,000 generations. 1,000,000 generations were discarded as burn-in and the rest were summarized to generate tree topology and posterior probability values. The 25 sequences from the Red Sea and the Indo-Pacific were deposited in Genbank (see Tables 2.1 and 2.2 for accession numbers).

The final 250 base pairs of sequence from the Red Sea *Symbiodinium*, corresponded to partial LSU sequence. They were aligned with LSU sequences downloaded from Genbank (Table 2.3) including the single instance of clade A from the WIO, exemplars of the temperate clade A known from the Mediterranean, and representatives from clades C and D. Two non-symbiotic *Gymnodinium* sequences were used as outgroup taxa and the reference sequences were aligned with the Red Sea sequences and the Indo-Pacific sequences using MUSCLE. The alignment was trimmed to the overlapping 238 base pairs and realigned. A neighbor joining branching diagram was used to compare genetic distance between the Red Sea tropical clade A sequences, the Indo-Pacfic tropical clade A sequences and the Mediterranean temperate clade A sequences. The Red Sea sequences were also aligned at the ITS2 locus with samples collected in the Mediterranean and evaluated for percent similarity.

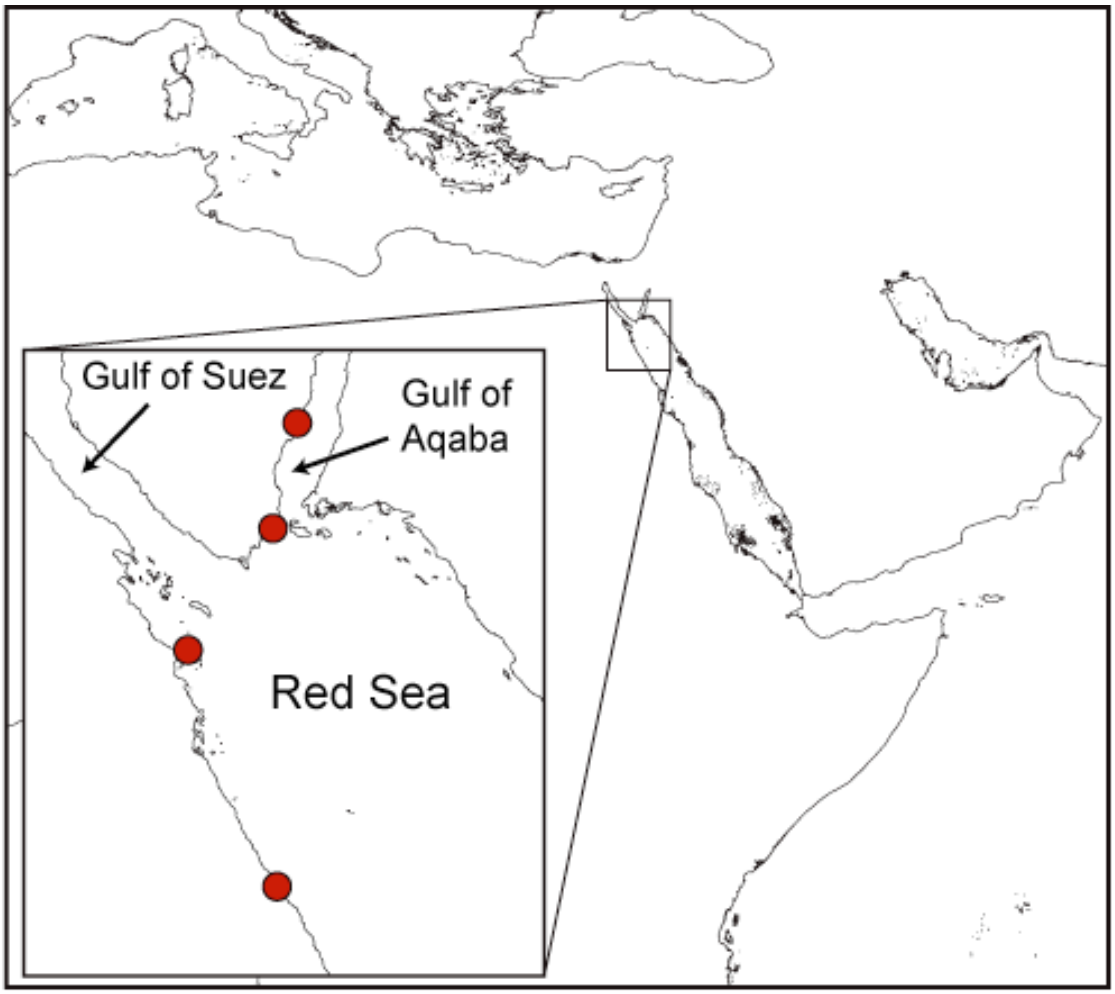

Figure 2.2 Sampling localities

Circles represent the four reefs where samples were collected along the Egyptian coast in the northern Red Sea. From North to South the localities were: Dahab, Ras Nasrani, Hurghada and El Qeseir. For details see Table 2.1.

Sample	Accession	Host	Location	Latitude	Longitude
DH.003	GU068991	*T. maxima*	Dahab	28.57203	34.53698
DH.006	GU069006	*T. maxima*	Dahab	28.57203	34.53698
DH.007	GU069005	*T. maxima*	Dahab	28.57203	34.53698
DH.008	GU069004	*T. maxima*	Dahab	28.57203	34.53698
DH.009	GU069003	*T. maxima*	Dahab	28.57203	34.53698
RM.008	GU068995	*T. maxima*	Ras Nasrani	28.57203	34.53698
RM.009	GU068984	*T. maxima*	Ras Nasrani	28.57203	34.53698
RM.010	GU068994	*T. maxima*	Ras Nasrani	28.57203	34.53698
RM.011	GU068993	*T. maxima*	Ras Nasrani	28.57203	34.53698
RM.012	GU068992	*T. maxima*	Ras Nasrani	28.57203	34.53698
HG.010	GU068990	*T. maxima*	Hurghada	28.57203	34.53698
HG.011	GU068989	*T. maxima*	Hurghada	28.57203	34.53698
HG.012	GU069002	*T. maxima*	Hurghada	28.57203	34.53698
HG.013	GU069001	*T. maxima*	Hurghada	28.57203	34.53698
HG.014	GU069000	*T. maxima*	Hurghada	28.57203	34.53698
HG.046	GU068999	*T. maxima*	El Qeseir	28.57203	34.53698
HG.047	GU068998	*T. maxima*	El Qeseir	28.57203	34.53698
HG.048	GU068997	*T. maxima*	El Qeseir	28.57203	34.53698
HG.049	GU068988	*T. maxima*	El Qeseir	28.57203	34.53698
HG.050	GU068996	*T. maxima*	El Qeseir	28.57203	34.53698

Table 2.1
Sample numbers, accession numbers and geographic coordinates for *Symbiodinium* from Red Sea giant clams.

ITS2 type	Accession	Host	Locality	Reference	Sample
C1	GU068982	*T. maxima*	Zanzibar	this study	Z.07.237
C1	GU068983	*T. maxima*	Zanzibar	this study	Z.07.170
A3	GU068985	*T. maxima*	Samoa	this study	OF.009
A3	GU068986	*T. maxima*	Moorea	this study	NS.002
A3	GU068987	*T. maxima*	Australia	this study	LI.083
A1	AF333505	*Cassiopeia*	culture	LaJeunesse 2001	
A2	AF333506	*Zoanthus*	culture	LaJeunesse 2001	
A3	AF333507	*Hippopus*	culture	LaJeunesse 2001	
A4	AF333509	*Plexaura*	culture	LaJeunesse 2001	
A5	AF333508	*T. squamosa*	culture	LaJeunesse 2001	
A6	AF186058	*Hippopus*	Philippines	Baillie et al. 2000	
C1	EU786002	*Amphisorus*	PNG	Fay et al. 2009	
C1	AF333515	*Rhodactis*	culture	LaJeunesse 2001	
Clade B	AF333511	*Aiptasia*	culture	LaJeunesse 2001	
Clade D	AJ311948	*Acropora*	Guam	Pochon et al. 2001	
Clade F	EU786036	*Amphisorus*	PNG	Fay et al. 2009	
Clade H	EU786028	*Amphisorus*	PNG	Fay et al. 2009	

Table 2.2
Accession numbers, host taxa and sampling locations for *Symbiodinium* from the Indo West Pacific included in the Bayesian phylogenetic analysis of ITS1-5.8S-ITS2-LSU (partial) rRNA. Samples from this study are in the top half of the table and reference sequences from the literature are in the bottom half.

Clade	Accession	Host	Location	Reference
Clade A	AY588453	*Acropora*	Kenya	Visram et al. 2006
Clade A	AY074949	*Condylactis*	Bermuda	Savage et al. 2002
Clade A	AY074953	*Stephanocoenia*	St. Croix	Savage et al. 2002
Clade C	AY588462	*Pocillopora*	Kenya	Visram et al. 2006
Clade C	AF170145	*Pavona*	Australia	unpublished
Clade C	AJ308893	*Acropora*	Reunion	Pochon et al. 2001
Clade D	AJ308900	*Pavona*	Guam	Pochon et al. 2001
A.med	AY074973	*Anemonia*	Italy	Savage et al. 2002
A.med	AY074974	*Anemonia*	France	Savage et al. 2002
A.med	AY588469	*Cereus*	Spain	Visram et al. 2006
A.med	AY588472	*Caryophyllia*	France	Visram et al. 2006
A.med	DQ865210	*Anemonia*	E. Med	Hunter et al. 2007
A.med	EU449046	*unknown*	E. Med	unpublished
Gymnodinium beii	AF060900	free-living	culture	Wilcox 1998
Gymnodinium simplex	AF060901	free-living	culture	Wilcox 1998

Table 2.3
Accession numbers, host taxa and sampling locations for *Symbiodinium* from the Indo-Pacific, the Caribbean Sea and the Mediterranean Sea included in the neighbor joining analysis of genetic distance in the first ~250 bp of LSU rRNA.

RESULTS

T. maxima from four sites in the Red Sea hosted symbionts that were 99.6% similar at the ITS1-5.8S-ITS2 rRNA locus and closely related to *Symbiodinium microadriaticum* (Freudenthal *sensu stricto)* (Figure 3). The five northernmost sequences collected in the Gulf of Aqaba near Dahab were identical to the ITS2 type A1 reference sequence, AF333505, isolated from *Cassiopeia xamachana* collected in Florida (LaJeunesse 2001). The other 15 Red Sea samples differed from AF333505 and the Dahab sequences at two positions within the ITS1 region but were identical within ITS2. These *Symbiodinium* sequences from Red Sea *T. maxima* showed no evidence of intragenomic variation.

The *Symbiodinium* sampled from *T. maxima* in Zanzibar hosted ITS2 type C1 *Symbiodinium* and the samples from Australia, Samoa and Moorea hosted ITS2 type A3 *Symbiodinium*. Clade C was well resolved and distantly related. The A3 sequences were approximately 95% similar to the two A1 genotypes from the Red Sea over the entire locus aligned in this study. Within ITS1 and ITS2, the A1 sequence (named following AF333505) varied by 24 single base pair changes from the A3 genotype (named following AF333507) collected in the Pacific and formed a well resolved sister clade.

No published *Symbiodinium* sequences from the Mediterranean Sea included the entire ITS1-5.8S-ITS2 rRNA locus. The ITS2 sequences from clade A collected in the Eastern Mediterranean Sea (DQ865210 and EU449046) differed from the Red Sea ITS2 sequences by more than 25% of the nucleotide positions within the fast-evolving spacer regions. Ribosomal large subunit sequences are much more conserved than the spacer regions in this locus because they code for proteins, which are under stabilizing selection. So while the ITS1 and ITS2 loci revealed fine-scale intraclade relationships, partial LSU could not distinguish between ITS2 types A1 and A3. However, although the tropical sequences from clade A were nearly identical across the 238 base pair region, the temperate symbionts from the Mediterranean were divergent enough within the partial LSU to form a separate clade (Figure 4). Approximately ten nucleotide differences separated the tropical ITS2 type A1/A3 clade from the temperate Mediterranean sequences. The resolution at this locus was insufficient to differentiate between ITS2 type A1 and ITS2 type A3 within the tropical clade but the single WIO clade A sequence (AY588453) clustered with the tropical clade and not with the temperate clade.

All of the Red Sea *T. maxima* hosted *Symbiodinium* sequences were virtually identical so the data did not support the hypothesis that symbiont type is ordered by depth and/or reef environment.

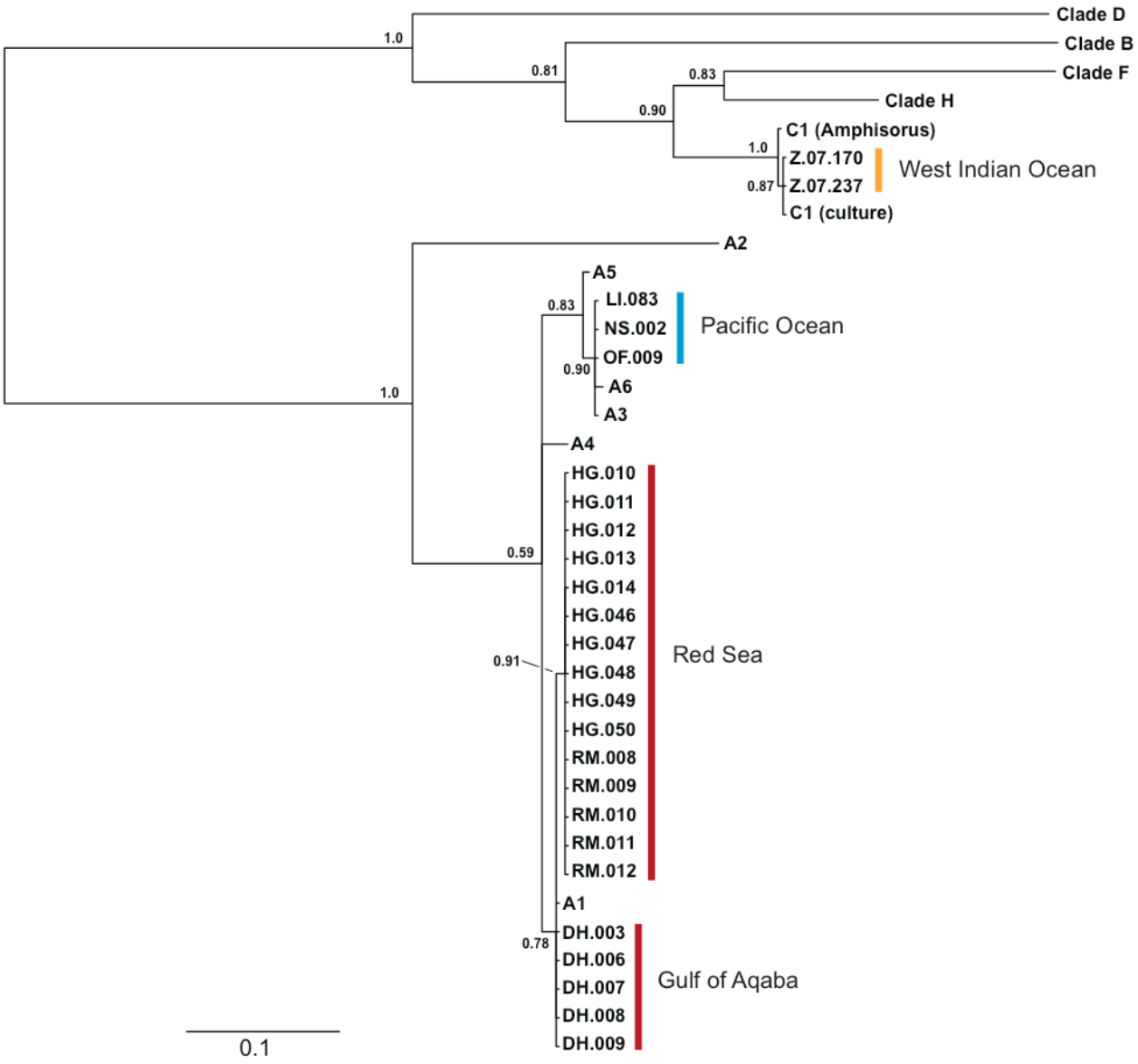

Figure 2.3 Phylogenetic systematics of *Symbiodinium* from *T. maxima*

Bayesian analysis of the ITS1-5.8S-ITS2 rRNA locus was used to infer a phylogenetic hypothesis for the relationships between *Symbiodinium* from the Red Sea, including the samples from Dahab in the Gulf of Aqaba, *Symbiodinium* samples from the Indo-Pacific and reference sequences downloaded from GenBank. Support values at each node are Bayesian posterior probability values. For accession numbers, host information and geographic coordinates, see Tables 2.1 and 2.2.

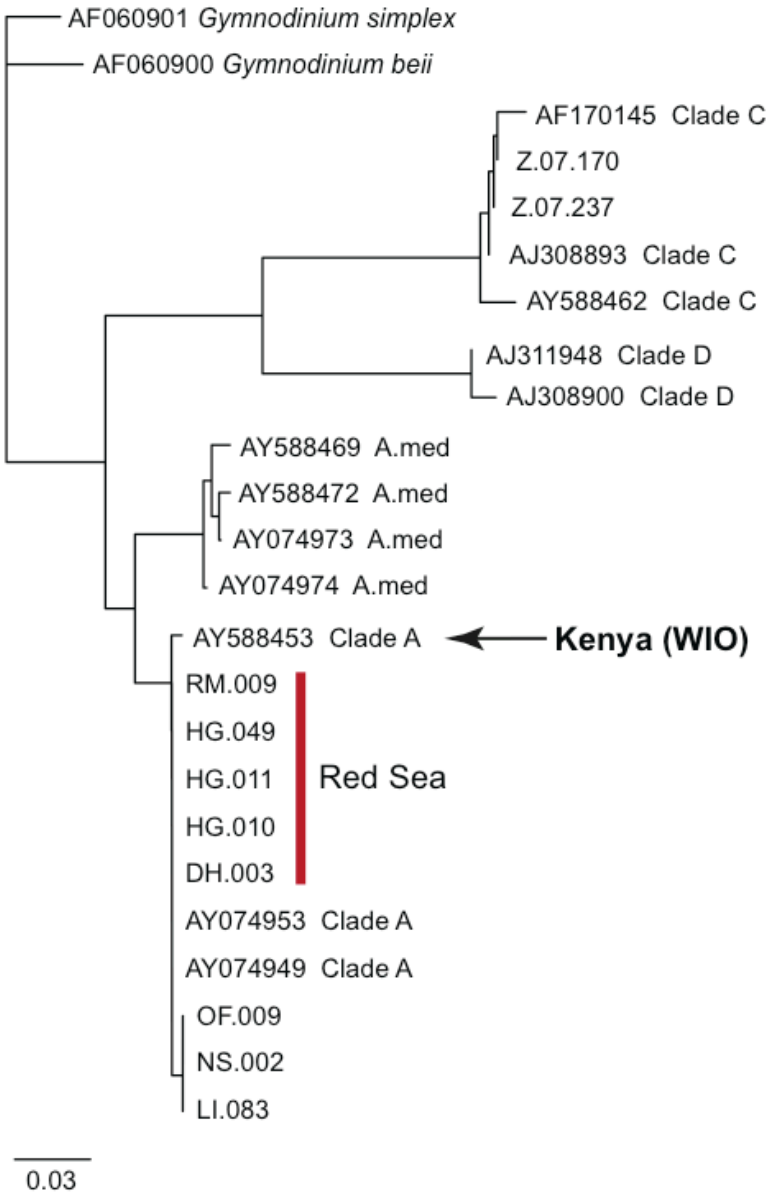

Figure 2.4 Neighbor-joining analysis of *Symbiodinium* partial LSU

Partial LSU was used to infer genetic distance between temperate clade A *Symbiodinium* collected from the Mediterranean Sea and tropical clade A, which included ITS2 types A1 and A3 as well as the other ITS2 types from clade A. Note that the single incidence of clade A collected in the WIO grouped with the Red Sea sequences and the other tropical sequences, lending support to the hypothesis that Red Sea *Symbiodinium* originated in the WIO. For accession numbers, host taxa and sampling location information see Tables 2.1 and 2.3.

DISCUSSION

Modern Red Sea *Tridacna maxima* populations originated in the West Indian Ocean

T. maxima is common in the Red Sea and along the east coast of Africa but absent from the modern Mediterranean Sea. The fossil record shows that ancestral tridacnid lineages originated in the Tethys Sea in the Eocene, 55 mya (Rosewater 1965; Harzhauser et al. 2008). During the late Miocene Messinian salinity crisis, the Atlantic connection to the Mediterranean closed, evaporation increased salinity and the basin eventually dried up. Tectonic activity eventually ruptured Gibraltar and the basin refilled but the Mediterranean Sea has not supported tropical reefs since the mid Miocene (Vennin et al. 2004; Braga et al. 2009; Govers 2009).

As the Arabian plate rotated and drifted northeast, the seaway in the Suez region closed isolating the Eastern Mediterranean Sea and the Northern Red Sea. Differences in the fossil faunas from the two regions date the closure to before the Messinian salinity crisis, when the Mediterranean appears to have evaporated, between 5 and 6 mya (Perrin et al. 1998; Plaziat et al. 1998). A shallow, Red Sea connection to the IWP broke open at the straits of Bab el Mandab in the early Pliocene and the Red Sea rift basin reflooded from the South (Coleman 1993). The European fossil record for tridacnids is uncertain because of early anthropogenic movement of shells but even if the Mediterranean was a refugium for *Tridacna* into the Miocene, the late Miocene drying would have caused extinction; moreover the Suez seaway was closed, preventing direct access to the Red Sea. Evaluation and clarification of the European fossil record indicated a 25 million year gap between Mediterranean tridacnid fauna in the late Oligocene and a livable Red Sea habitat (Harzhauser et al. 2008). Lack of tropical fauna in the Mediterranean concurrent with Red Sea rifting, closure at Suez and subsequent Pliocene flooding from the south indicated that modern Red Sea *T. maxima* populations originated in the WIO and entered through Bab el Mandab (Rosewater 1965; Taviani 1998).

Periodic Ice Ages have lowered sea level and restricted circulation between the Red Sea and the Indian Ocean over the last five million years. Intense evaporation increased salinity causing intermittent planktonic extinctions in the Red Sea (Siddall et al. 2003). During the last glacial maximum (LGM), northeastern Africa experienced an arid phase and sea level dropped 120 meters (Figure 2). Evaporation raised the salinity to approximately 55 ppt (Hemleben et al. 1996). Giant clams are susceptible to increasing salinity and larvae die when exposed to high values (Tan and Yasin 2000). Diatoms, dinoflagellates and other plankton are absent from sediment cores from this period (Hemleben et al. 1996; Fenton et al. 2000; Siddall et al. 2003; Fernandes et al. 2006). Some speculation that fresh water input may have maintained lower salinities in the Gulf of Aqaba exists but currently there is no evidence of less saline refugia (Fenton et al. 2000; Arz et al. 2003). Under extremely salty conditions, free-living dinoflagellates and many reef organisms including *T. maxima* and other *Symbiodinium* hosts must have also gone locally extinct (Taviani 1998).

When circulation resumed and oceanographic conditions stabilized after the LGM, giant clams and other tropical colonists from the WIO reentered through Bab el Mandab and founded modern Red Sea reef communities. Red Sea reefs are continuous around the perimeter of the rift basin, facilitating dispersal of larvae. Mature giant clams spawn several times per year and their larval stage lasts approximately 9-11 days (Heslinga and Fitt 1987). Broadcast spawning on this scale makes them one of the most fecund marine invertebrates (Heslinga and Fitt 1987) and enhances their dispersal across the IWP (Benzie and Williams 1997; Nuryanto and Kochzius

2009). These lines of evidence support the hypothesis that *T. maxima* reinvaded and recolonized the Red Sea within the last 12,000 years.

Modern Red Sea *T. maxima* and their A1 symbionts are an endemic holobiont

T. maxima from the Red Sea consistently and exclusively hosted A1 symbionts. Giant clams are horizontal transmitters; they acquire symbionts as larvae at each generation (Rosewater 1966), but despite the ability to acquire and maintain other symbionts, all Red Sea *T. maxima* sequenced hosted A1 *Symbiodinium*. An early report documented both clade A and clade C in *T. maxima* from the Pacific but did not use the ITS2 marker to determine subclade identity (Baillie et al. 2000b). While reported from other Red Sea hosts, such as corals and jellyfish (LaJeunesse 2001; Pochon et al. 2001), the A1 lineage has not yet been observed in *Tridacna*.

Symbiodinium ITS2 type A1 is a basal lineage within clade A (Correa and Baker 2009). It is a generalist and it is present in diverse hosts from many geographic regions (LaJeunesse et al. 2004; Stat and Gates 2008; LaJeunesse et al. 2009). The ITS2 type A1 *Symbiodinium* documented from the Red Sea was collected in the Gulf of Aqaba at one of the northern-most reefs in the world (LaJeunesse 2001; Pochon et al. 2001). Zooanthid hosts from high latitude reefs around Taiwan and Japan also hosted ITS2 type A1 *Symbiodinium* (Reimer et al. 2006). These occurrences are consistent with the hypothesis that generalists are more likely to tolerate extreme conditions such as high latitude environments.

If most Red Sea biota went extinct at the LGM, then modern Red Sea reefs are relatively young (Taviani 1998). As sea level rose following the LGM, *T. maxima* larvae from the WIO moved back through the Bab el Mandab and repopulated Red Sea reefs. Subsequently ITS2 type A1 *Symbiodinium* infected and colonized the new *T. maxima* population. The unique association between the host and the tolerant, generalist symbionts survived the extreme temperature and salinity regime and persists today. This endemic holobiont evolved within the last 12,000 years, and, in time, perhaps additional lineages will diversify and/or specialize in Red Sea *T. maxima*.

Why is only one symbiont phylotype present in *T. maxima*?

Several alternative hypotheses potentially explain why *T. maxima* in the Red Sea exclusively hosted A1 *Symbiodinium*. A species is present in a particular place either because (1) only one species colonized and it remains because of historical legacy or (2) the modern environment selected survivors from an initial, pandemic population (Martiny et al. 2006). But in the case of symbiosis, either environment or host can impose selection; therefore, three alternative hypotheses can account for the exclusivity of this partnership.

1. Only one lineage of *Symbiodinium* with the potential to live in clam tissues successfully invaded the Red Sea and the ITS2 type A1 population documented here is descended from that single founder population of dinoflagellates. Thus the isolation and geological history of the Red Sea prevented colonization by additional symbiont strains.

2. Multiple strains entered the Red Sea but selection imposed by the extreme environment eliminated competing lineages and only the A1 type persisted in *T. maxima*.

3. Multiple strains entered the Red Sea but selection imposed by the host organism, *T. maxima,* eliminated competing lineages and only the A1 type persisted.

Because other symbiont lineages are available from reservoirs in alternative hosts, such as foraminifera, octocorals and corallimorpharians (Pochon and Pawlowski 2006; Goulet et al. 2008; Kuguru et al. 2008), the first hypothesis was rejected; A1 is not the only lineage of *Symbiodinium* available to infect the clams in the Red Sea. The third hypothesis is also rejected because *T. maxima* outside of the Red Sea hosted other symbiont clades, for example, the individuals collected from Zanzibar for this study hosted ITS2 type C1 (for additional symbiont diversity in giant clams see: Baillie et al. 2000b; Baker 2003). The second hypothesis is therefore the simplest explanation: multiple lineages of symbiont arrived in the Red Sea, including those that persist in alternative hosts, and were available to colonize the *T. maxima* population, but the environment selected for partnering with ITS2 type A1. Selection imposed by abiotic conditions and biotic interactions such as competition between symbiont lineages may interact to limit potential partnerships but these possibilities could not be independently tested here. As the *T. maxima* – A1 *Symbiodinium* holobiont evolves in isolation, its success may have further impeded host colonization by additional symbiont strains despite their availability.

Where did the *Symbiodinium* A1 population originate?

We infered that *T. maxima* in the Red Sea originated in the WIO, but the origin of the symbiont population is less obvious because there is no fossil record for *in hospite Symbiodinium*. Free living, planktonic dinoflagellates did not survive high salinities associated with Pleistocene glaciations (Siddall et al. 2003; Siddall et al. 2004); therefore, three alternative origin hypotheses could explain the arrival of the *Symbiodinium* lineage A1 in the Red Sea ~12,000 years ago.

1. The symbionts and the clams colonized as a unit. The holobiont combination migrated into the Red Sea from the WIO but subsequently went extinct on the east African coast.

2. The free-living symbionts originated in the modern Mediterranean Sea and entered the Red Sea through the Suez Canal. Upon arrival, they colonized giant clam hosts, replacing ancestral strains.

3. The A1 symbionts, either as free-living individuals in the Indian Ocean or as symbionts associated with alternative hosts of WIO origin, entered the Bab el Mandab and then infected the clams, replacing whatever symbiont strain the founding clam population hosted in the WIO.

Alternative one: **Hosts and symbionts colonized together; both originated in WIO.**

This hypothesis argued that the *T. maxima* – A1 *Symbiodinium* holobiont arrived intact as settling larvae drifted into the Red Sea from the WIO. *T. maxima* larvae acquired and maintained their native WIO symbiont populations throughout immigration and dispersal up the Red Sea coastal reef system over successive generations. Under these circumstances, clams from coastal East African reefs would also host ITS2 type A1 symbionts or a closely related lineage derived from A1.

This hypothesis was tentatively rejected because clams from the East African coast did not host ITS2 type A1, but rather C1 symbionts (Figure 3) or other symbionts from clade A (Chapter 1). *Symbiodinium* from clade C form a derived lineage with significant nucleotide substitutions easily distinguishable with only partial LSU (Figure 4) and comparable to order-level divergences between non-symbiotic lineages of dinoflagellates (Rowan and Powers 1992). A few *T. maxima* individuals from East Africa and many from other parts of the IWP, including Australia, Samoa and Moorea, hosted ITS2 type A3. Both C1 and A3 are common lineages found in reef dwelling organisms and have often been documented in *Tridacna* populations (Baillie et al. 2000a; LaJeunesse 2001; LaJeunesse et al. 2009). A3 and A1 are both ancestral members of clade A (Correa and Baker 2009) but they form resolved clades at the ITS1-5.8S-ITS2 rRNA locus (Figure 3). The hypothesis that the holobiont arrived intact was rejected based on the lack of ITS2 type A1 symbionts identified from *T. maxima* in the WIO. However, the original *T. maxima* – A1 *Symbiodinium* combination might have gone extinct in the WIO after dispersing into the Red Sea. If this idea can be tested with newly available data, or if the *T. maxima* – A1 *Symbiodinium* holobiont is described from the WIO, this hypothesis will be resurrected.

Alternative two: Modern symbiont populations originated in the Mediterranean.

The second alternative hypothesis proposed a recent colonization and replacement by A1 symbionts originating in the Mediterranean Sea and entering through the Suez Canal when it was cut 150 years ago. Although this is an extremely recent event, considerable shipping traffic suggests the possibility that *Symbiodinium* could travel through the canal into the Gulf of Suez to colonize at least the northern Red Sea.

To test the Mediterranean origin alternative, tropical clade A symbiont sequences found in Red Sea *T. maxima* populations were compared to temperate clade A sequences from Mediterranean host organisms. Although none of the published temperate clade A sequences utilized the same locus (Savage et al. 2002; Visram et al. 2006), separately comparing ITS2 and partial LSU sequences rejected a Mediterranean origin for the Red Sea symbionts. Clade A symbionts from *Anemonia* sp. collected in the eastern Mediterranean Sea and the Red Sea sequences differed at more than 25% of the nucleotide positions within the ITS2 locus. At the highly conserved partial LSU locus, the temperate clade A symbionts differed by approximately 10 base pair substitutions from the tropical clade A lineage, which included ITS2 types A1 and A3 (Figure 4). This constitutes enough divergence at this locus in the Mediterranean clade A symbionts to warrant a unique subclade designation. Of the ten cnidarian species sequenced from the Mediterranean, none hosted tropical clade A *Symbiodinium*. In addition, only one polyp of one anemone species hosted clade B symbionts. These data indicated that overall *Symbiodinium* diversity in the Mediterranean Sea is low and therefore, despite limited sampling, it is unlikely that tropical clade A *Symbiodinium* in the Mediterranean has been missed.

Finally, coral hosts introduced to the Caribbean system from the Indo West Pacific maintained their nonnative symbionts when surrounded by alternative types over 35 years and the ITS2 type harbored in these corals had not spread to other Caribbean hosts (LaJeunesse et al. 2005). These introduced symbiont populations were viable and host specific for decades suggesting that the Suez Canal was cut too recently to affect a complete symbiont repopulation in giant clams across the northern Red Sea. Even if A1 is discovered north of Suez, it will be important to distinguish whether Mediterranean origin dinoflagellates migrated into the Red Sea or whether they migrated from the Red Sea into the Mediterranean. Lack of tropical clade A

symbionts and low overall *Symbiodinium* diversity in the Mediterranean Sea, as well as evidence for the maintenance of nonnative, specific symbionts over decadal time scales tentatively rejected the hypothesis that ITS2 type A1 symbionts in modern Red Sea giant clams, originated in the Mediterranean.

Alternative three: Modern symbiont populations originated in the Indian Ocean but arrived independently from the clam hosts.

The third alternative hypothesis is that both host and symbiont ancestors entered through Bab el Mandab but were not originally associated as a holobiont. The symbionts arrived independently in alternative hosts or as free-living dinoflagellates. The results from this study did not find ITS2 type A1 *Symbiodinium* in *T. maxima* from Zanzibar. WIO reefs have predominantly yielded symbionts from clades C and D (Baker 2004; Visram and Douglas 2006; Sebastian et al. 2009). Approximately 140 corals from 21 sites along the coast of Mozambique and South Africa hosted *Symbiodinium* from clades C and D (Sebastian et al. 2009). Along the Kenyan coast only *Symbiodinium* clades C and D were found in corals (Baker 2004). Thirty-six other Kenyan coral colonies from 7 sites yielded symbionts predominantly from clades C and D (Visram and Douglas 2006).

One clade A *Symbiodinium* sequence (AY588453) was found in four *Acropora valida* colonies but because only the large subunit sequences were published, the symbionts could not be identified at the ITS2 level. However, partial LSU sequence indicated that the Kenyan symbionts grouped with the tropical clade A and not with the temperate clade A symbionts (Figure 4). Symbionts from clade A were observed in only 7 % of *Tridanca* from the east coast of Africa but all were closely related to phylotype A3 (Chapter 1). Although phylotype A1 has never been reported from the WIO, the presence of tropical clade A symbionts in hosts from reefs along the African coast suggested a WIO origin for the tropical clade A symbionts in the Red Sea giant clams.

Overall diversity of *Symbiodinium* hosts on WIO reefs suggests that the likelihood of finding a source of A1 in the WIO is high compared to the Mediterranean where host diversity is limited (Hughes et al. 2002). In the ten Mediterranean hosts that have been sampled, the divergent, temperate clade A *Symbiodinium* dominated with a single exception; one sample hosted clade B symbionts (Savage et al. 2002; Visram et al. 2006). The coral taxa *Stylophora*, *Acropora* and *Millopora* that hosted ITS2 type A1 *Symbiodinium* in the Gulf of Aqaba are common in the WIO (LaJeunesse 2001; Pochon et al. 2001). This evidence supported the hypothesis that the ITS2 type A1 symbionts in Red Sea *T. maxima* originated in an alternative WIO host.

CONCLUSIONS

Five principal lines of evidence supported an independent WIO origin for both host and symbiont followed by subsequent colonization and modern persistence of an endemic holobiont in the Red Sea: (1) WIO *T. maxima* hosted ITS2 type C1 or A3 symbionts, not A1, (2) the organisms living on modern Red Sea reefs, that hosted phylotype A1, have WIO origins, (3) WIO reefs are host-diverse and other WIO taxa host additional symbiont diversity, (4) a divergent lineage of temperate *Symbiodinium* almost exclusively dominated the low diversity of Mediterranean host organisms, (5) The Suez Canal was cut very recently. While recovery from

coral bleaching describes moderately flexible symbioses over ecological scales, these data suggested that symbiosis between marine invertebrates and their dinoflagellate endosymbionts is also flexible over evolutionary time scales. In order to colonize a changing ocean system, an endemic holobiont evolved via separate dispersal events by hosts and symbionts.

Implications for the evolution of an endemic holobiont

Although the host-*Symbiodinium* symbiosis is usually considered a mutualism, recent evidence suggested that clade A may be a parasitic lineage and still share some traits with its free-living ancestors. Dinoflagellates collected from coral reef sand nest within clade A (Hirose et al. 2008), suggesting that this group, which is basal to other *Symbiodinium* lineages, can also live independently of hosts. When compared to other more derived lineages of *Symbiodinium*, clade A contributed less photosynthate to the host (Stat et al. 2008). Another experiment showed that jellyfish larvae forced to select novel symbionts from the environment at each generation (horizontal transmission) evolved more parasitic symbionts and had lower fitness than a sibling population of the same hosts that maintained cooperative symbionts via vertical transmission (Sachs and Wilcox 2006). Increasingly, phylogenetic analysis reveals examples of mutualism breakdown within symbiotic lineages though evolutionary time (for relevant examples see Wilcox 1998; Van Oppen et al. 2005). Some partnerships revert to parasitism as an intermediate step between mutualism and a non-symbiotic lifestyle (Sachs and Simms 2006).

As variation in sea level alters the world's marginal seas in the long term and/or humans change the environment in the short term, holobiont breakdown may become more common. After this breakdown, individual partners disperse, and hosts may re-associate with relatively more infectious, parasitic lineages, which allow the holobiont to quickly occupy new niches. On a geologic time scale the intermediate stage is evident as an endemic holobiont which subsequently may go extinct, break down into non-symbiotic species if the symbiont substantially reduces holobiont fitness (Wilcox 1998; Van Oppen et al. 2005), or dominate in the new habitat, as shown in this study. As conditions change or new habitat becomes available, holobionts may revert to partners that are generally less cooperative but allow them to more effectively compete for niche space in extreme environments like the Red Sea.

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CHAPTER 3

High symbiont diversity and unusual host-symbiont combinations in modern regions of low host diversity: Historical biogeography of the Tridacnidae – *Symbiodinium* holobiont

ABSTRACT

The modern center of marine biodiversity is the Central Indo West Pacific (IWP). This region is home to more species of fish, corals and giant clams (Tridacnidae) than any other part of the tropical ocean. Global marine diversity declines to the east, into the central Pacific Ocean and to the west, into the Indian Ocean. I showed that dinoflagellate endosymbionts (*Symbiodinium*) in giant clams were distributed along a similar longitudinal diversity gradient but a secondary center of biodiverisity for *Symbioinium* was in the West Indian Ocean (WIO). I identified six species of giant clam and eight symbiont lineages from Papua New Guinea and Australia in the Central IWP. Symbiont diversity in giant clams was limited to two or three lineages in most other regions and host species were specific for certain symbionts. Although only two clam species lived on reefs in three localities in Kenya and Zanzibar, they hosted six lineages of *Symbiodinium*. *Tridacna maxima* associated with all six phylotypes identified in the WIO.

Tridacna usually hosted ancestral generalist lineages that were also common in a variety of alternative hosts. *Symbiodinium* from clams in the WIO included these ancestral types but in addition, one of the ITS2 types recovered from *T. maxima* in Kenya was not known from other *Tridacna* populations. Two of the WIO phylotypes were not otherwise observed outside of the Central IWP. The Indo-Pacific biodiversity gradient predicted depauperate reefs 8,000 km from the center of marine biodiversity. To explain rare combinations of hosts and symbionts and rare symbiont types found distant from the center of marine biodiversity, I evaluated ecological and historical hypotheses that would support increased diversity on the coastal reefs of East Africa. Patterns in the holobiont biogeography suggested that high diversity in the WIO was a relict of host diversity in that region that developed in the Miocene and shifted into the Central IWP in the Pliocene. Although all but two host lineages are now extinct in the WIO, diverse symbionts persisted within the former range and associated with *T. maxima* producing rare combinations and suggesting that holobiont distributions shift more slowly than independent partner lineages.

INTRODUCTION

Symbionts evolve to cooperate in low productivity environments and increasing available nutrients increases the abundance of less cooperative strains (Johnson 1993; Denison and Kiers 2004; Thrall et al. 2007). Low nutrient availability in tropical seawater limits the efficiency of filter-feeding heterotrophs and has selected for cooperative associations between reef dwelling host organisms and diverse microbes (Knowlton and Rohwer 2003; Coffroth and Santos 2005; Thurber et al. 2009). Dinoflagellates from the genus *Symbiodinium,* associate with a variety of marine phyla providing additional energy resources to their host and facilitating calcification. The hosts provide access to nutrients and a safe environment where symbionts can live in high densities. Coral reefs are one of the most diverse ecosystems on earth and the relationship between coral hosts and their photosynthesizing symbionts sustains the reef as the energetic basis of a complex food web and creates a complex physical structure that forms habitat for other organisms.

Symbiodinium evolution

Symbiodinium associates with hosts from foraminifera, porifera, cnideria and mollusca. Once thought to be a single species, *Symbiodinium microadriaticum* (Freuenthal *sensu strictu*), research consistently shows that diverse lineages within the genus occupy different hosts and different ecological niches. At least eight divergent clades of *Symbiodinium* are currently recognized and each includes multiple subclade level lineages with varying distributions, ecologies and physiologies (Freudenthal 1962; Taylor 1973; LaJeunesse et al. 2004b; Coffroth and Santos 2005).

The true history of the dinoflagellate lineage *Symbiodinium* is more difficult to reconstruct because *in hospite* dinoflagellates do not fossilize. Accepting the necessary limitations on interpretation, a relaxed molecular clock suggested that the genus *Symbiodinium* began diversifying in the Eocene (Pochon et al. 2006) concurrent with the first appearance of Tridacnidae (Harzhauser et al. 2008). Clade A is the most basal lineage of *Symbiodinium* and the ancestors of clade A were the first to adopt a symbiotic lifestyle 50 million years ago (mya) followed by substantial diversification in the Oligocene and the Miocene (Pochon et al. 2006). The crown group, clade C, is the most diverse lineage of *Symbiodinium* and the most common symbiont in modern marine invertebrates, including tridacnids, across the Indo West Pacific (IWP). Two primary, ancestral clade C symbiont genotypes radiated into numerous host specific and regionally specific types and this branch diversified extensively, beginning in the middle Miocene, as the global marine climate was warming (Zachos et al. 2001; LaJeunesse 2005).

Symbiodinium lineages exhibit varying degrees of specificity depending on geography, abiotic factors and mode of transmission. Host organisms that pass their symbionts on to their offspring as part of an egg package or brood and release larvae maintain the strictest specificity. Horizontal transmitters release gametes in mass spawning events and are less specific; however, many can transmit their symbionts via asexual reproduction. Coral fragmentation and cell division in foraminifera consistently pair the same symbiont genotypes with the same host genotypes. Dinoflagellate generation time is short and populations turn over many times inside a host, so even for organisms that horizontally transmit symbionts, reciprocal adaptation, may be possible for periods of time between sexual reproduction events. While some *Symbiodinium* lineages are broadly distributed generalists, hosts from a particular region, characterized by consistent environmental factors, often associate with specific *Symbiodinium* lineages (Baker

2003; LaJeunesse et al. 2004b; Pochon et al. 2004; Garcia-Cuetos et al. 2005; Stat and Gates 2008; LaJeunesse et al. 2009).

The Tridacnidae lineage

Basal giant clam ancestors, the genera: *Goniocardium* Vasseur, 1880, *Avicularium* Gray 1853, and *Byssocardium* Munier-Chalmas 1882, evolved 55 to 50 million years ago in the Tethys Sea. Derived from the family Cardiidae and adapted to a symbiotic lifestyle, many lineages had intermediate morphologies that included increasing size, anatomical rotation to facilitate basking and byssal opening to allow anchoring (Stasek 1962; Rosewater 1965; Schneider 2002; Harzhauser et al. 2008). As continental rifting opened the Red Sea and created the Arabian plate, divergent lineages of tridacnid clams and other mollusks evolved and populated the newly created shallow seas in the Middle East (Harzhauser et al. 2007; Harzhauser et al. 2008).

By the early Miocene, ancestral clam populations in the Tethys had gone extinct and the African-Arabian province was the center of biodiversity for giant clams as they diverged into two genera, *Tridacna* and *Hippopus*, and then shifted into the IWP province (Harzhauser et al. 2007; Harzhauser et al. 2008; Renema et al. 2008). Other marine faunas including other mollusks and scleractinian corals simultaneously diversified and shifted from an Eocene center of biodiversity in the Tethys, to an Oligocene center of diversity in the African-Arabian province. In the early Miocene these groups established another center of biodiversity in the Central IWP, which remained after the African-Arabian faunas went extinct (Fukami et al. 2004; Harzhauser et al. 2007; Renema et al. 2008).

As Arabia collided with Anatolia at the end of the Miocene and the majority of the shallow seas were uplifted and disappeared, the center of biodiversity for mollusks and corals as well as giant clams completely shifted into the Indian Ocean and the Central IWP (Vermeij 2001; Harzhauser et al. 2007; Renema et al. 2008). The modern genus *Tridacna* was first recorded from the Central IWP at the end of the Miocene, where newly formed shallow seas resulted from rising sea levels. The extant genus *Hippopus* first appears in the fossil record in the late Miocene in the Central IWP. But there are anecdotal accounts of older fossils and significant morphological and genetic evidence infers that it must have diverged from the stem group in the early Miocene (Rosewater 1965; Schneider and Foighil 1999; Hall 2002; Hoeksema 2007; Harzhauser et al. 2008). At the end of the Pleistocene several lineages of *Tridacna* went extinct outside the Central IWP (Rosewater 1965; Crame 1986; Harzhauser et al. 2008). For the last 5 million years the coral triangle has been the center of biodiversity for the family Tridacnidae (Harzhauser et al. 2008).

***Symbiodinium* in Tridacnidae and other hosts in the WIO**

Compared to cnidarians and foraminifera, giant clams evolved symbioses with *Symbiodinium* relatively recently. Tridacnidae hosted a small fraction of the diversity of symbiont types known from alternative host taxa and the most common phylotypes were ancestral, generalist types also known from other hosts (Chapter 1). Corals and foraminifera house their intracellular symbionts enclosed in unique membranes but giant clams maintain *Symbiodinium* populations in tertiary tubules that develop from digestive system organs (Norton et al. 1992). Giant clams often hosted symbionts from clade A, a basal lineage of *Symbiodinium* that cultures easily in the laboratory and donates fewer energetic resources to its host (Chapter 1; Ishikura et al. 2004; Stat et al. 2008)). Morphologic and isotopic evidence from fossils showed that Triassic corals from the Tethys realm hosted algal symbionts approximately 250 mya

(Stasek 1962; Stanley and Swart 1995; Harzhauser et al. 2008). The giant clam family did not even appear in the fossil record until the Eocene and even if the basal lineages hosted symbionts, the symbiosis still evolved 200 my later than corals. These observations supported the hypothesis that clams recently evolved photosymbiosis.

Modern Tridacnidae is distributed across the Indo-Pacifc and like many marine taxa, the modern center of biodiversity is the Central IWP (Rosewater 1965; Harzhauser et al. 2008). Genetic diversity for the species *Tridacna crocea* is also highest in the coral triangle (DeBoer et al. 2008; Kochzius and Nuryanto 2008). Clade C symbionts were most common in *T. squamosa* across the IWP. Although they hosted *Symbiodinium* from clades A, C and D, lineages within clade A were the most common symbionts in *T. maxima* across the IWP. However, along the east coast of Africa, *T. maxima* hosted mostly clade C symbionts, unlike other regions in the IWP. Clams from the Central IWP hosted fewer clade C *Symbiodinium* than the WIO and clade C was not present in clams from reefs in the Central South Pacific (Chapter1).

Only six publications reviewed *Symbiodinium* genotypes information for cnidarian hosts in the West Indian Ocean and they described limited symbiont diversity (Burnett 2002; Baker et al. 2004; Visram and Douglas 2006; Goulet et al. 2008; Macdonald et al. 2008; Sebastian et al. 2009). Many studies used RFLPs to fingerprint symbiont types or sequenced rRNA to identify symbiont clade but not the ITS2 subclade level lineages. These papers also reported the dominance of clade C along the African coast. *Symbiodinium* from clade D was found in three studies and clade A was identified once (Baker et al. 2004; Visram and Douglas 2006; Sebastian et al. 2009). Data on symbiont diversity from the WIO is extremely limited when compared to the number of publications that focus on *Symbiodinium* in a variety hosts from the Pacific Ocean (ex. Australia) or the Caribbean Sea (ex. Bahamas).

Holobiont biogeography defined

A lineage evolves to occupy a particular niche where both history and ecology interact to determine the modern distribution. Within the context of symbiosis, the species distributions of hosts and symbionts are inter-dependent. The distribution of a host, such as *Tridacna*, is dependent on its own history, which if it has a hard shell or skeleton, may be reasonably well represented in the fossil record. Host distribution may also be influenced by the historical biogeography of its obligate symbionts. However, symbionts are less tractable in the fossil record and molecular evidence must be used to infer their biogeographic patterns, which may also depend on both their own niche requirements and those of their hosts. Host physiology determines part of the symbiont niche, forcing the symbiont to adapt as the host adapts through time. Historical processes that individually affected the dispersal of either partner would explicitly affect the distribution of the holobiont.

Evolution of a holobiont depends on the evolutionary trajectory of each partner, which may not be entirely reciprocally determined. The holobiont may also evolve along its own trajectory and develop unique biogeography. Patterns in holobiont diversity, or the distribution of combinations of partners, are not independent of the processes that affect each partner; however, processes that are unique to the symbiosis also shape holobiont distributions and leave characteristic patterns. Examples include selection mosaics generated by reciprocal interactions between partners or the remixing of traits (particularly traits associated with the symbiosis) between populations of hosts and/or symbionts (Thompson 2005). This may include horizontal transfer of genes between populations in the case of bacterial symbionts (Silva et al. 2005). Symbiosis can expand (or retract) what would be the limited realized niche of either partner

alone (Bena et al. 2005; Thrall et al. 2007) imposing distinct patterns on the biogeography of the association. Cooperative associations evolve towards parasitism in regions where productivity is high and/or habitat complexity is high but relationships are more cooperative in regions of low productivity or low complexity (Hochberg and van Baalen 1998; Klironomos 2003; Thrall et al. 2007). Biogeographic patterns generated by these processes and others associated with the holobiont can be evident when the distribution of possible combinations of partners is investigated on a regional scale. Biogeography of the holobiont may obscure the story if the maintenance of the relationship and the distribution patterns are only considered from the perspective of each individual partner or worse yet, a single partner. In order to explain the distribution of the modern relationship between two individual partners, we must consider biogeography of the host, the symbiont and the holobiont and the unique forces that affect each.

The historical biogeography of Tridacnidae is relatively clear because mollusk shells fossilize easily (Schneider 2002; Harzhauser et al. 2008). However the historical biogeography of the Tridacnidae-*Symbiodinium* association is subject to many more influences than just host distribution. *Symbiodinium* biogeography can be analyzed via phylogenetic systematics and databases that document their presence across regions and host taxa (LaJeunesse et al. 2004a; Pochon et al. 2006; Pochon et al. 2007). Although specificity patterns have been interpreted within a geographic model of coevolution (LaJeunesse et al. 2004b; Thompson 2005; LaJeunesse et al. 2009), the concept of holobiont biogeography has not been explored in *Symbiodinium* systems. Here I considered geological, ecological and historical hypotheses with respect to questions regarding diversity and unique distributional patterns in giant clam-*Symbiodinium* holobiont biogeography.

MATERIALS AND METHODS

I collected giant clam mantle tissue and sequenced *Symbiodinium* from across the IWP. In Chapter 1, I discussed the symbiont sequences from *T. maxima* and *T. squamosa* and patterns with respect to host specificity and environmental parameters. Here I sequenced symbionts in additional giant clams species from Papua New Guinea and Australia at the center of coral reef biodiversity including: *T. derasa*, *T. gigas*, *T. crocea* and *Hippopus hippopus* (for localities see Figure 3.1). These giant clam species do not live outside the Central IWP and Western Pacific today although fossils indicated a wider distributional range as recently as the Pleistocene (Crame 1986; Harzhauser et al. 2008).

Giant clam mantle tissue was cut from the edge of the mantle by SCUBA divers and preserved in 80% ethanol as soon as possible. The tissue was stored at -4 degrees until it could be transported to the University of California Museum of Paleontology where it was extracted using Qiagen extraction kits. The extract was amplified using primers S_DINO and L_O (Pochon et al. 2001) and the template was sequenced at the University of California, Berkeley Sequencing Facility following the same methods described in Chapters 1 and 2.
The sequences were blasted against the NCBI database to determine preliminary identity. They were aligned using MUSCLE (Edgar 2004) and trimmed to ~730 base pairs that included partial SSU–ITS1–5.8S–ITS2–partial LSU. Representative sequences from each clade were downloaded from GenBank and aligned with the trimmed sequences. *Gymnodinium* was used as an outgroup because it appears to be the sister group to *Symbiodinium* (Shaked and de Vargas 2006). Phylogenetic positions of the clades within the *Symbiodinium* genus were inferred using

MrBayes (Huelsenbeck and Ronquist 2001) following the procedure described in Chapter 1. Because of long-branch attraction as a result of diverse spacer regions within the locus, the sequences were trimmed to the ~250 nucleotide ITS2 portion and aligned to other sequences within each major clade to more accurately infer evolutionary relationships between closely related phylotypes within the lettered clade level lineages. Ten phylotypes were identified in the giant clam samples based on the phylogenetic inferences (Chapter 1, for trees see Figures 1.2 through 1.5; for complete list of samples and locality information, see also Appendix: A.3.1 and A.3.2).

To determine if there was a longitudinal diversity gradient, the numbers of holobionts, hosts and symbionts from each geographic location were plotted against longitude where they were collected relative to PNG to represent distance from the center of biodiversity. As an outlier, East African symbiont diversity was compared to *Symbiodinium* populations from the Central IWP. In order to understand why diversity was so high in the WIO, multiple hypotheses were evaluated with the support of additional evidence from diverse fields including geology, paleontology, oceanography and ecological niche theory.

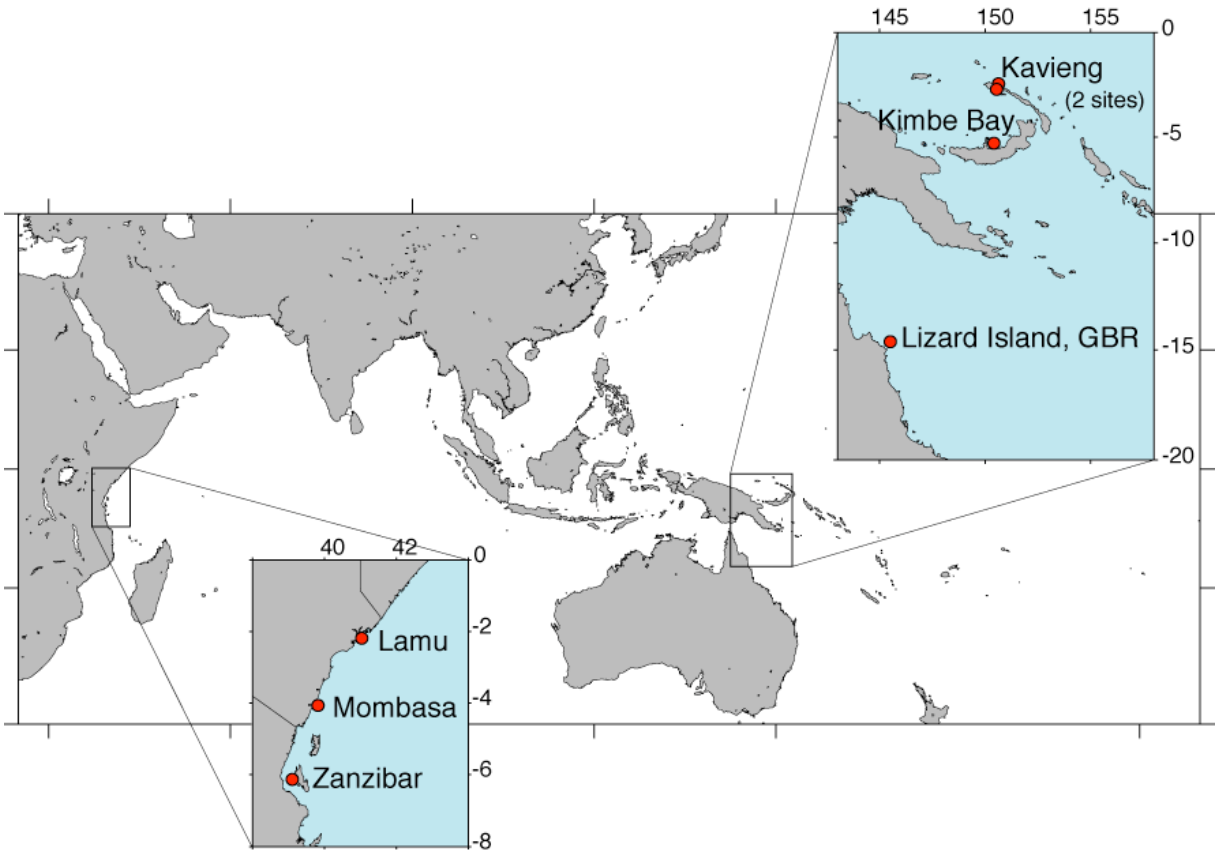

Figure 3.1 Sampling localities in the centers of biodiversity

Symbiodinium samples from giant clam mantle tissue were collected along the East African coast and 4 sites from the Central Indo West Pacific. In the WIO I collected from Lamu and Mombasa in Kenya and Zanzibar. From the central IWP, I collected samples from the Great Barrier Reef in Australia, and Kimbe and 2 sites in Kavieng, Papua New Guinea. For geographic coordinates see tables A.3.1 and A.3.2 in the Appendix.

RESULTS

Clam and *Symbiodinium* diversity declined across their IWP distribution at longitudes moving away from the coral triangle to the east. However, when I compared symbionts and holobionts from across the IWP, a secondary center of biodiversity for clam – *Symbiodinium* holobionts along the East African coast was clearly apparent (Figure 3.2). Six clam species hosted eight ITS2 types and 23 unique holobiont combinations were observed from three Central IWP localities, Kavieng and Kimbe Bay in Papua New Guinea and Lizard Island, Australia. Thirty six clam individuals collected in Kenya and Zanzibar yielded 2 host species, *T. maxima* and *T. squamosa,* and 6 symbiont ITS2 types (Chapter 1). In these 2 hosts from these 3 localities, I observed 7 holobiont combinations. *T. maxima* associated with all six *Symbiodinium* lineages and *T. squamosa* associated with ITS2 type C1. One of the symbionts from the WIO and two of the holobionts were unique to this region and were not observed in the Central IWP (Figure 3.3).

I identified ITS2 type C1 in all host lineages from multiple locations across East Africa and the Central IWP. *T. squamosa* from East Africa only hosted ITS2 type C1 and it was found in 74% of *T. maxima* (Chapter 1). Five other *Symbiodinium* phylotypes were identified in *T. maxima* from sites along the East African coast: ITS2 types A3, A3a, A4, C66 and D1. All of these types were also identified in clams from PNG and Australia except for A4, which was only found in East Africa. Phylotype C1 was found in only 21% of the Central IWP hosts where *T. squamosa* also hosted ITS2 types D1, A3x and A3a. Phylotype C2 was unique to the Central IWP and although C66 was sequenced from *T. crocea* and *T. gigas* from Australia and PNG, in East Africa it associated with *T. maxima* (Appendix: A.3.1 and A.3.2).

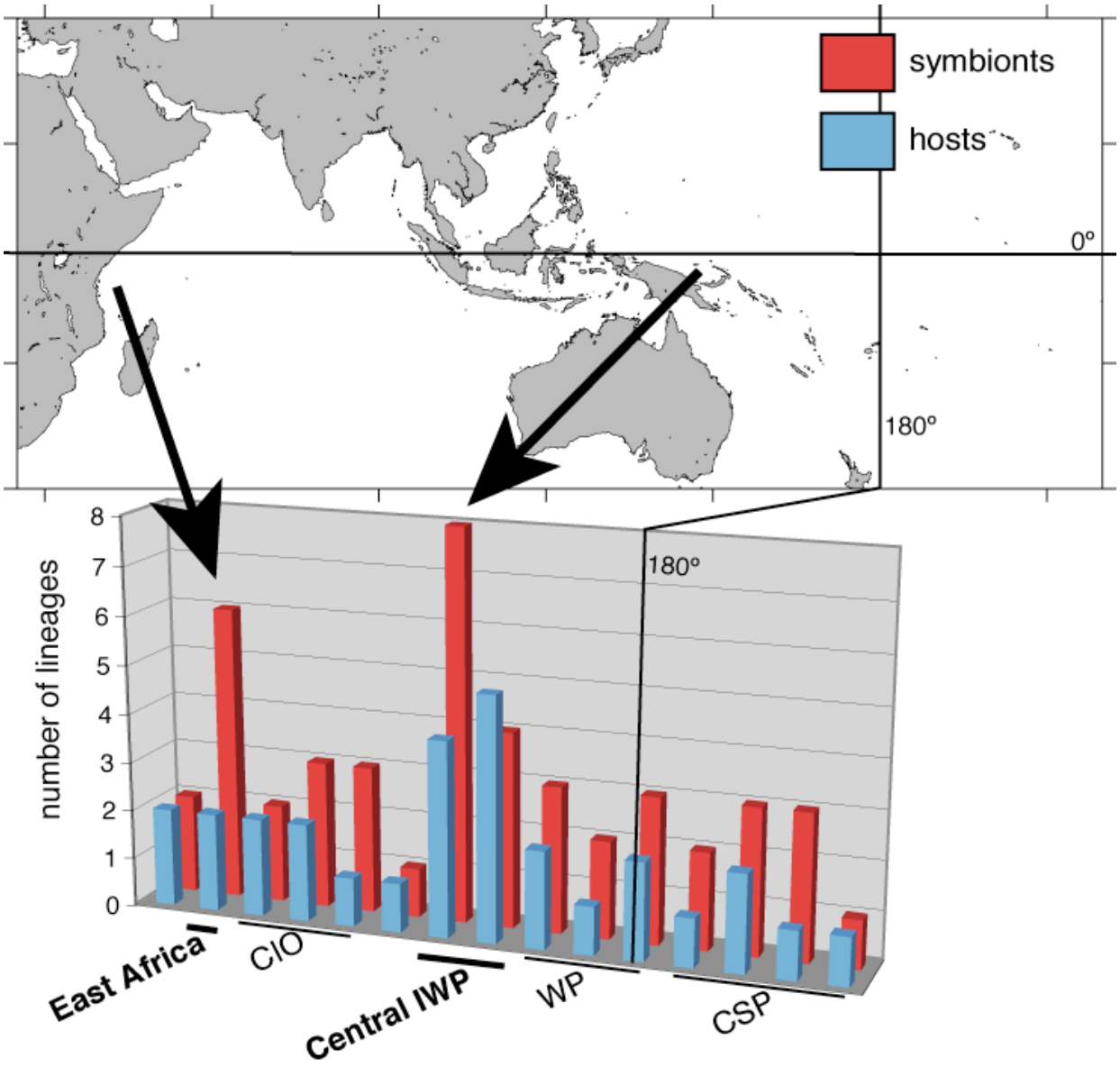

Figure 3.2 Centers of biodiversity

The plot shows the number of lineages at each locality across the Indo West Pacific. The bars are arranged from the Red Sea in the East on the far left to Moorea in the West on the far right. East Africa includes localities in Kenya and Zanzibar and Central IWP includes localities from PNG and Australia (Figure 3.1). CIO = Central Indian Ocean, WP = Western Pacific and CSP = Central South Pacific. The 180° longitude line, opposite the Prime Meridian, runs through Fiji. Black arrows denote centers of biodiversity. For hosts (blue), number of lineages is greatest in the Central IWP but for symbionts (red), there are two peaks: one in the Central IWP and the other along the East African coastline in the WIO. Data from Chapter 1 (see Appendix).

DISCUSSION

Tectonic history and implications for diversity in the two regions

The Central IWP region has been a particularly active tectonic zone for the last 25 million years. This area is a junction between four major plates: the Indian-Australian Plate, the Eurasian Plate, the Philippine Plate, the Pacific Plate. As the Australian Plate continually moves north, it is subducting under the Eurasian Plate, cracking to form new micro-plates and collecting the small fragments of continental crust that include Indonesia and shoving them into Southeast Asia. The tectonics of this region are complex and although there have been multiple reconstruction attempts, questions remain (Hall 2002).

Tectonic activity alters coastlines and shifts islands forcing reef organisms to adapt as their habitat rises, sinks, shifts and buckles. During the Pleistocene, low sea level stands rearranged coastlines and virtually closed the connection between the Pacific Ocean and the Indian Ocean. The lowest sea level stands exposed the Sahul and Sunda shelves and their reefs and created extended coastlines (Hall 1998). New islands and shallow seas potentially contributed to increased rates of speciation by isolating some populations; however, uniform tropical latitudes and geographic complexity also maintained diversity through time. Diversity can be strongly correlated to both habitat heterogeneity and complexity and this region has encompassed diverse, complex habitats over the last 25 million years, even as their relative positions shifted (Hall 2002; Hoeksema 2007).

In addition to complicated geology and changes in sea level, reef organisms in the Central IWP also had to survive alterations in prevailing current patterns. When environmental conditions change and organisms are not longer adapted to their current habitat, selection pressures will drive them extinct if they do not disperse into more suitable habitat. However, changing current patterns could compromise finely tuned dispersal mechanisms and jeopardize a population's migration potential. Paleo-currents have been reconstructed multiple different ways within the Central IWP, suggesting that moving water through this complicated zone has undergone multiple different phases in recent history (Gordon and Fine 1996; Hall 1998; Hall 2002). Islands in the Central IWP were appearing, disappearing and shifting via tectonic activity and as a result, the current regimes were not stable.

Aside from distribution shifts, shallow water, benthic organisms in the Central IWP are predominantly affected by local current patterns. But broad scale changes associated with geologic change may have altered small scale local patterns, which would affect populations of sessile organisms in diverse biotic and abiotic ways. Water temperature over the reef changed depending on where new currents originated and how fast they moved. The organisms may have physiological constraints and be unable to tolerate changes in temperature or salinity. Many reef organisms are filter-feeders and as currents patterns varied, they may not have successfully accessed appropriate planktonic food resources. Reproduction patterns and larval dispersal were affected. For many reef taxa, changing currents would have affected the mixing of gametes during mass spawning events. If enough larvae were not carried over appropriate habitat within a certain developmental window, the population would go extinct (Vermeij 2001). Altering the temperature and flow regimes would force organisms to tolerate a new set of biotic and abiotic conditions, adapt through time or go extinct.

In contrast to the turbulent geology and oceanography of the Central IWP, the eastern edge of the African continent is a passive margin and has been relatively stable over the last 20 million years. The African Plate is splitting along the inland Rift Valley to the west of the

coastline. To the north, seafloor spreading in the Red Sea is separating the Arabian Plate from the African Plate. In the middle of the Indian Ocean at the Central Indian Ridge, the African Plate is pulling away from the Indian-Australian Plate. Movements at these plate boundaries do not directly affect elevations or create volcanic activity along the East Africa coastline, which remains stable over the middle of the African Plate in contrast to the active geology under the Central IWP.

Sea level changes in East Africa were gradual and primarily due to climate change. Continuous coastlines made migration to appropriate habitat possible and allowed populations to shift up and down the coastline. Current patterns remained consistent along the East African coastline (Smart et al. 2007; Gourlan et al. 2008) and consistent oceanographic conditions implied that dispersal mechanisms and thermal tolerances of local organisms were not affected by changing flow regimes bringing water of different temperatures and origins over the coastal reefs.

How do geology and oceanography affect diversity?

Symbiodinium hosts are most diverse in the Central IWP, the global center of marine biodiversity and marine diversity declines along a longitudinal gradient away from this region (Paulay 1997; Hughes et al. 2002; Harzhauser et al. 2008). Reef organisms are dependent on warm, well-lit waters and grow around islands and along continental coastlines in tropical latitudes. Increased tectonic activity in the Central IWP, where multiple plates are colliding, has produced extensive, shallow water habitat where reefs have proliferated and diversified over the last 20 million years. Although mechanisms that generated diversity remain unclear and hypotheses about the region functioning as a cradle or museum remain inconclusively tested, diversity patterns show that more species of plants, larger foraminifera, fish and marine invertebrates live on Central IWP reefs than anywhere in the ocean (reviewed in: Paulay 1997; Wilson and Rosen 1998; Hughes et al. 2002; Hoeksema 2007; Renema et al. 2008; Bellwood and Meyer 2009).

The diversity gradient for giant clams also peaks in the Central IWP. Both the literature on giant clam distributions and my sampling scheme recorded more species of *Tridacna* in Australia and Papua New Guinea than anywhere else in the range of modern giant clams. Although *Symbiodinium* diversity has not been exhaustively databased on a global scale, in this study, six symbiont lineages were identified from giant clams on the East African coast and seven were collected from giant clams collected in the Central IWP. The intermediate locations were less diverse (Figure 3.2). Because these results would not be predicted by hypotheses that described the center of biodiversity in the Central IWP (Paulay 1997; Hughes et al. 2002; Hoeksema 2007; Reaka et al. 2008), I explored the question: Why do symbiont and holobiont diversity patterns differ from host biodiversity patterns?

High levels of biodiversity in the Central IWP have been attributed to complicated geography in the region. Turbulent geologic history altered oceanographic systems and created diverse habitats that selected for a variety of phenotypes; therefore, lineages diversified in the Central IWP because of variation in the selection landscape. By the same argument, relatively stable geology and limited environmental complexity would limit diversification of East African reefs. East Africa is fringed by extensive reef systems but for many taxa they are depauperate relative to the center of marine biodiversity (Vermeij 2001; Hoeksema 2007; Williams and Duda 2008). In contrast to the Central IWP, East Africa has experienced relatively stable geology and

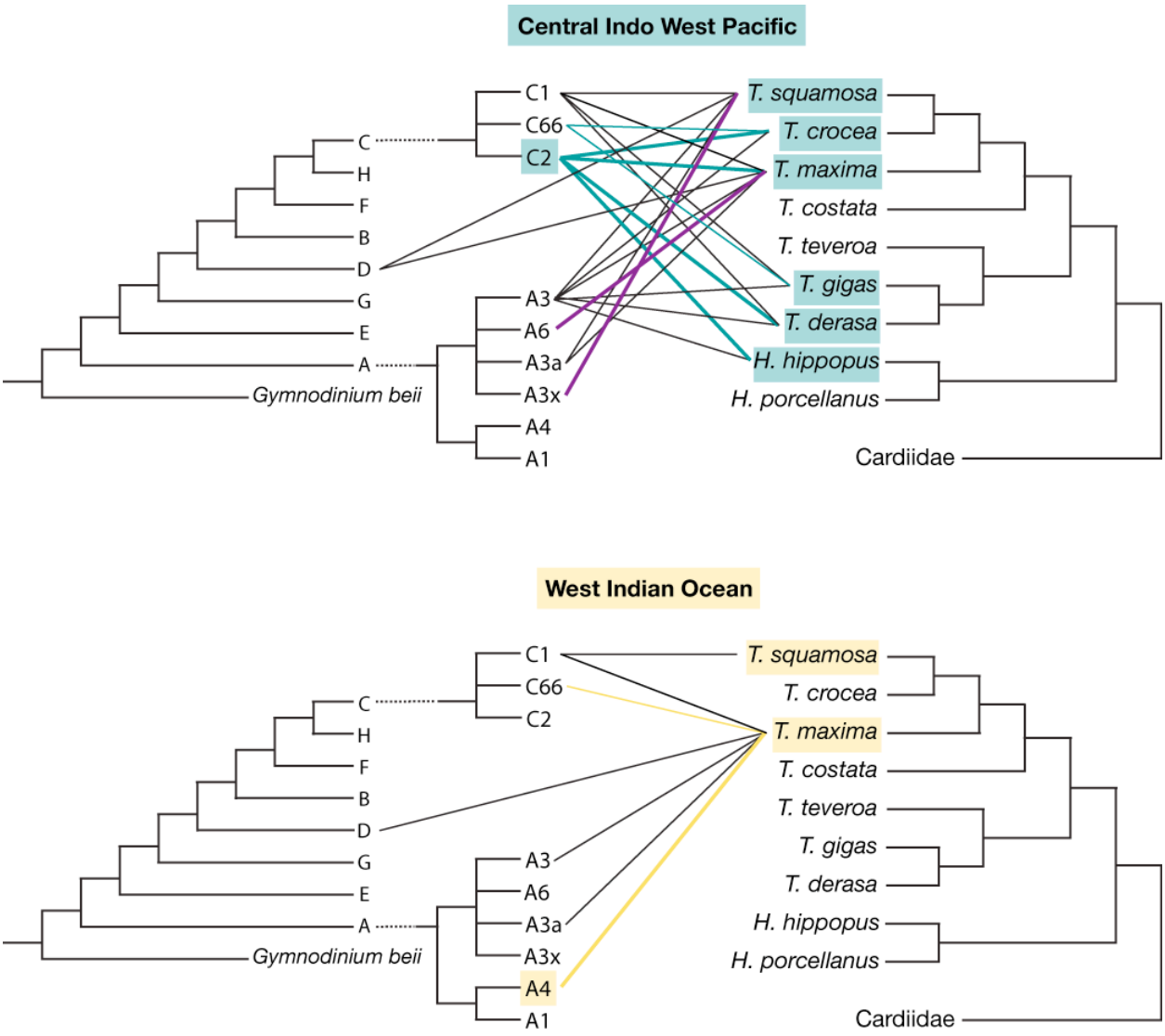

Figure 3.3 Holobionts from the Central IWP and West Indian Ocean

Samples from the Central Indo West Pacific were collected in four localities in Australia and Papua New Guinea and samples from East Africa (West Indian Ocean) were collected in three localities from Kenya and Zanzibar (Figure 3.1). *Symbiodinium* lineages are shown on the left and hosts from Tridacnidae are shown on the right (cladograms based on (Stat et al. 2006) and(Schneider and Foighil 1999)). I sampled six host species from the Central IWP and only two from the WIO, but high symbiont diversity was identified in both regions. A4 was unique to the WIO and C2 was unique to the Central IWP although it was found in four host species there. C66 was hosted by *T. maxima* in the WIO but in the Central IWP it was hosted by *T. gigas* and *T. crocea*. A6 and A3x (purple) were common in the Pacific Ocean, rare in the Central IWP and not present in the WIO. Although *T. squamosa* was a generalist in the Central IWP, it was specific for C1 in the WIO. *T. maxima* hosted diverse symbionts in both regions.

oceanographic circulation patterns over the last 25 million years (Gordon and Fine 1996; Saher et al. 2007; Smart et al. 2007; Gourlan et al. 2008). Other taxa were depauperate in the WIO including Tridacnidae. However, these data indicated that WIO reefs were habitat for diverse *Symbiodinium* lineages and holobionts despite limited hosts as habitat. Six symbiont lineages associated with only two giant clam species (Figure 3.3).

If the two regions are so different geologically, but exhibited similar symbiont diversity patterns, then geologic history alone cannot account for the IWP center of biodiversity and alternate hypotheses should be evaluated. Although the historical biogeography of giant clams is fairly well documented (Schneider 2002; Harzhauser et al. 2008), the evolution of the symbiosis, which includes both host and symbiont lineages, must be considered in order to explain patterns in the distribution of holobionts throughout the hosts' range. The history of combinations of host and symbiont lineages, must be examined, individually from and complementary to the history of the individual partner lineages. Here I compared and contrasted geological history, evolutionary history and niche ecology for two taxa that together form a symbiosis with characteristic distribution patterns in order to evaluate five alternative hypotheses about the evolution of holobiont diversity. Patterns in the bigeography of holobionts were attributed to a combination of historical processes that affected both partners.

West Indian Ocean diversity, patterns and hypotheses

Giant clam–*Symbiodinium* diversity data supported the hypothesis that host biodiversity is highest in the Central IWP. However diverse lineages of symbionts were identified in association with clams along the East African coast and unusually diverse combinations of *T. maxima* holobionts existed well outside of the predicted range for high diversity values. Five hypotheses addressing sampling biases, geology, oceanography, ecological niche space and historical biogeography potentially explained this divergence from the larger IWP biodiversity pattern:

1. Sampling bias. Resources have limited research in the WIO and conservation practices affected the survival of clam populations in many localities.
2. Efficient dispersal along consecutive shallow water habitat promoted diversity along the fringing reefs on the East African coast.
3. East Africa is a passive margin with limited tectonic activity. Stable oceanographic conditions have not substantially disrupted reef systems in the WIO. Consistent current patterns and temperature regimes allowed diverse combinations to survive.
4. Mutualism models predict reduced competition and relaxed specificity when partners do not have to protect themselves from "cheater" lineages. Less productive combinations were out-competed by specialists in the Central IWP but in the WIO, less competitive combinations persisted.
5. The African-Arabian province was the center of biodiversity in the late Oligocene/early Miocene. Holobiont and symbiont richness along the African coast is a historical legacy of Miocene diversity, most of which has shifted east into the central IWP.

1. Sampling bias

I described diverse symbiont populations from *Tridacna* along the East African coast and from the Central IWP. Many models of coral reef biodiversity showed the Coral Triangle in the

Central IWP as the center of marine biodiversity and predicted depauperate coral reefs in the WIO (Paulay 1997; Hughes et al. 2002; Hoeksema 2007). Following modern distribution patterns in other organisms, reefs in the Central IWP would be expected to house most of the clam and symbiont diversity; however, these data showed that East African clams housed diverse symbiont communities in low abundance.

The Indian Ocean is disproportionately under-sampled. Historically, research scientists preferred certain field sites for logistical, political and financial reasons. Many reef biologists, based in Australia, focus their studies on the Great Barrier Reef (GBR). Field stations available across the Pacific Ocean are valuable resources and promote reef research (Wyman et al. 2009) but there are only a few field stations in the Indian Ocean. I am excited about the expanding literature on *Symbiodinium* from the Indian Ocean (Baker et al. 2004; Visram and Douglas 2006; Goulet et al. 2008; Macdonald et al. 2008; Sebastian et al. 2009) but sequences available on GenBank for comparative analyses are limited. Many research groups used low resolution loci to document molecular diversity and identified organisms into only the largest groups. Studies on corals in the Indian Ocean sequenced many fewer symbionts at loci appropriate to divulge subclade identity when compared to the body of research from the GBR. *Symbiodinium* from the WIO have been identified to clade but many studies did not utilize ribosomal spacers or chloroplast genes that would have resolved fine scale lineage diversity within major clades. Lineages divergent at finer scales are ecologically unique and it is difficult to summarize functional and/or geographic diversity of host-symbiont partnerships based solely on clade level data (LaJeunesse 2001; Sampayo et al. 2007; Frade et al. 2008).

The sampling regime used to survey symbiont diversity in Tridacnidae was biased by fishing and conservation. For example, in PNG, large clams were under heavy fishing pressure and therefore the population was approaching local extinction. Large species such as *T. derasa* and *T. gigas* were difficult to locate. These large, shallow-dwelling clams are protected on the GBR and this was one of the only localities where I could consistently sample from diverse species within Tridacnidae.

Fewer studies in the WIO means that limited sampling could conceal cryptic host diversity. In 1991 a new species of giant clam was described from Tonga and in 2008 a new species was described from the Red Sea (Lucas et al. 1991; Richter et al. 2008). Although giant clams are conspicuous members of the reef community, because there has been less work in the Indian Ocean, it is possible that cryptic endemics remain to be described.

2. Continental fringing reefs facilitate dispersal

Dispersal for many benthic reef taxa is dependent on the larval phase in the life cycle. Organisms travel long distances in the water column as plankton before developing and settling sessile or reef associated benthic adults. Continental coastlines along the east coast of Africa provided a consistent source of shallow marine environments that are not isolated by large expanse of open ocean like island reefs in the Pacific or Central Indian Oceans.

Isolated islands are extreme examples of dispersal dependent ecosystems where density and size of neighboring reefs determines potential sources and sinks of genetic diversity (MacArthur and Wilson 1967). A taxon's ability successfully to shift its distribution in time with changing conditions is contingent on the dispersal abilities of the taxa and variation in the dispersal traits for individuals in the population. If conditions change and there is no nearby available habitat within the limit of dispersal ability, the populations would go extinct but

organisms on continental fringing reefs can disperse along the coastline or to higher ground. As sea level rose and fell, populations could shift up and down as well as along coastlines seeking favorable habitat more easily than between islands. *Tridacna* fossils are common in Pleistocene reef outcrops along the East African coast (Crame 1986; Schneider 2002). This fossil evidence indicated that East African reefs were able to keep pace with rising sea levels and build new reefs in the flooded shallows or potentially retreat to deeper water at low sea level stands. The consistent availability of shallow water habitat along coastlines allowed efficient distributional shifts as environmental conditions changed. Lack of selection on dispersal ability may have increased *Symbiodinium* diversity by allowing holobionts to survive periods of changing sea level by shifting their distribution.

If continental reefs were inherently more diverse because they provided consistent sources of refuge shallow water habitat as sea level changed and didn't require finely tuned dispersal mechanisms, in addition to high diversity in the WIO and the Central IWP, I would have also observed diverse communities in the Red Sea and Sri Lanka; both are continental coastlines with fringing reefs. However these regions exhibited limited diversity: Red Sea *T. maxima* were specific for *Symbiodinium* ITS2 type A1 and in Sri Lanka, *T. maxima* was specific for *Symbiodinium* ITS2 type A3 (Chapter 1; Chapter 2). These continental fringing reefs were minimally diverse much like reefs around remote Pacific Islands, suggesting that that proximity to continental coastlines, was not the only factor contributing to increased diversity of *Symbiodinium* and/or holobionts in the WIO.

3. Stable geological setting and consistent oceanographic conditions

The Central IWP has been implicated as a center of diversification and a center of accumulation for diverse lineages from across the IWP because of its geographic complexity (of the many: Pianka 1966; Briggs 2007; Halas and Winterbottom 2009). It has been tectonically unstable and changing conditions constantly selected for tolerant combinations of hosts and symbionts and selected against poorly adapted combinations since the beginning of the formation of the Central IWP 25 mya. Since the WIO has experienced limited geologic activity and stable oceanographic regimes over the last 20 million years, the region may have allowed the persistence of less tolerant combinations, thus acting as a center of accumulation for intolerant holobionts or holobionts with lower fitness.

As the Indian-Australian Plate drifted north, variable tectonic activity created and destroyed shallow marine environments in the central IWP (Hall 1998; Hall 2002). A variety of diverse habitat options were consistently available to reef organisms because shallow, flooded shelves along continental coastlines existed in close proximity to multitudes of islands. As conditions changed, populations evolved, migrated and/or shifted their ranges but because of the complexity of the region, there was always proximal, suitable refuge habitat. Compared to isolated island systems in the South Pacific or Indian Ocean, the complex islands and seas in the Central IWP served as a sink of available habitat for immigrants from other regions, even when conditions were unstable. This idea is the basis of a series of accumulation hypotheses, which implicated the region as a museum of diversity. Low atolls could end up completely submerged in high sea level stands. During Pleistocene low sea level stands, shallow lagoons and continental shelves (ex: SE Asia) dried completely. As sea level dropped and reefs were exposed, many taxa went extinct, but some organisms dispersed to reefs around new islands or along the new coastlines (Hall 2002; Renema et al. 2008). Populations may go extinct in other

regions because they cannot evolve fast enough to survive changing conditions. However, if members of the population could successfully disperse to new habitat, they may have migrated to the Central IWP where diversity was preserved in the complexity of the region. The prevalence of diverse habitat options through time in a complex region is only one line of evidence used to explain high biodiversity in the Central IWP (of the many: Pianka 1966; Rosen 1988; Briggs 2007; Halas and Winterbottom 2009). When refuge habitat existed within the dispersal range of a taxon, extreme conditions favored the organisms that could disperse to new reefs. If they were isolated in a new region, additional diversity was generating by allopatric speciation but often, when oceanographic patterns changed due to underlying geologic activity, dispersal abilities could not keep pace and populations went extinct on remote reefs (Paulay and Meyer 2006).

Although the Central IWP potentially accumulated diversity because habitat complexity served as a refuge, shifting oceanographic conditions may have dampened the effect. Most of the reefs in the Central Indian Ocean and the South Pacific formed around atolls or islands where changing sea level could drastically vary the amount of available shallow, near-shore environment and suitable reef habitat. The larval phase of the life cycle depends on currents to transport the next generation of benthic organisms to their habitat. Marine organisms on reefs around islands are reliant on effective dispersal strategies to adjust their ranges as conditions change. In regions with complex oceanography or shifting current patterns through time, like the Central IWP (Gordon and Fine 1996), the survival of a lineage would be dependent on dispersal mechanisms. In a stable region like the WIO, the survival of a lineage would not be as dependent on dispersal abilities and diversity would accumulate as poor dispersers went extinct in other regions as a result of changing conditions.

Host-symbiont partnerships are vulnerable to changing abiotic factors that are also affected by current patterns. On the ecological time scale reef symbioses are particularly sensitive to changing temperatures. Evidence from observations of bleached organisms suggested that individual partners are less susceptible to temperature variation than the combination of host and symbiont living as an associated unit. When exposed to high temperatures, corals bleached under-performing symbionts or intolerant symbionts abandoned hosts. Subsequent to a bleaching event, hosts survive provided they reacquired symbionts within a limited period of time. Symbionts survive (either free-living or in alternative hosts) and are capable of re-colonizing hosts if conditions stabilize (Thornhill et al. 2006; Kuguru et al. 2008; Baskett et al. 2009). If changes in oceanographic patterns altered temperatures, salinities and other qualities of the water that flows over reefs, benthic organisms would have to adapt rapidly. However, many organisms have narrow tolerances and bleach easily (Hughes et al. 2003; Fabricius et al. 2004; Hoegh-Guldberg et al. 2007). If the changing conditions caused hosts to bleach their symbionts, the community would rely on flow regimes to redeliver new symbionts to repopulate bleached hosts. Bleaching would be more common under changing conditions and redelivery of appropriate symbionts would be less consistent in regions where underlying geology is unstable and current patterns are variable.

As a stable marine environment over the last 25 million years, the East African coast may have served as a different kind of center of accumulation for *Symbiodinium* populations. Because stable habitat was consistently available and dispersal was facilitated by continuous coastline and consistent current regimes, perhaps the WIO was a refuge habitat for diverse modern holobionts because it served as a sink for intolerant but functional combinations of hosts and symbionts. In the Central IWP, tectonic activity shifted islands, altered current patterns and changed temperature regimes, and while these processes may have generated diversity as

organisms adapted to new conditions they also selected against intolerant symbioses. Symbiotic relationships would breakdown and holobionts would go locally extinct if harsh conditions continued to select against vulnerable combinations. Variation in the community and selection for association between novel combinations of partners would produce populations of competitive, resilient holobionts that could tolerate the changes. If geologic activity did not substantially disrupt either elevation or current patterns, as in the WIO, selection for tolerant holobionts would be less and additional diversity would be conserved. Associations between marginally compatible partners would persist as small populations until unfavorable environmental conditions eliminated them.

As plates shifted and sea level changed, complex habitat availability generated diversity but rates of evolution for component taxa would be higher in active tectonic zones like the Central IWP because the intolerant lineages went extinct. If stable reef structures and oceanographic conditions on the East African coast allowed competitively inferior symbioses to persist, the same combinations of host and symbiont lineages in areas of more intense change, like the Central Indo-Pacific would not be observed in the WIO. In Australia and PNG, I found *T. squamosa* and *T. maxima* in symbiosis with ITS2 type C1 and ITS2 type D1 indicating that these combinations were reasonably resilient. However, symbiont phylotype, A4 was found in a single *T. maxima* individual in East Africa (Figure 3.3). Because it was not present in the Central IWP, the ITS2 type A4 – *T. maxima* holobiont population may be a low abundance, unstable combination that is selected against in the Central IWP. Or perhaps A4 is a less efficient symbiont for giant clams and in other regions selection limits it to alternative hosts such as corals.

ITS2 type C66 was observed in *T. crocea* and *T. gigas* in the Central IWP but in the WIO it colonized *T. maxima* (Figure 3.3). *T. maxima* is present in the Central IWP, but if strong selection eliminated the ITS2 type C66 – *T. maxima* holobiont or prevented association between those two partners in the Central IWP, that *Symbidoinium* phylotype may have been limited to other hosts including *T. crocea* and *T. gigas.* Although I could not test the effects of geography on host specificity for phylotype C66 because the range of *T. crocea* and *T. gigas* does not extend to East Africa, I could infer that the ITS2 type C66 – *T. maxima* holobiont is an unstable combination because the host is present in the Central IWP as is the symbiont, but they did not associate.

The A4 – *T. maxima* holobiont and the C66 – *T. maxima* holobiont are examples of combinations that were less tolerant and therefore eliminated by turbulent conditions in the Central IWP. If unstable holobionts were contributing substantially to diversity in the WIO, I would expect to see more distinct combinations but most of the symbionts and combinations were present in both localities so this effect is probably minimal. In Kenya and Zanzibar these rare holobionts may represent less tolerant combinations that survived in low abundance because of the less stressful environmental conditions. Although the number of unique combinations did not contribute substantially to the inflated WIO diversity, these data offer preliminary support and the hypothesis could be further tested.

Explaining diversity in the WIO with an accumulation hypothesis would suggest certain phylogenetic patterns and certain patterns in holobiont functional diversity. Lineages in centers of accumulation should on average be older than lineages from surrounding, less stable regions because diversity has built up through time (Rocha et al. 2008; Bellwood and Meyer 2009; Halas and Winterbottom 2009). Evidence of older holobionts would support the accumulation hypothesis but it remains unclear what category of data could be used to determine the age of the

association since symbionts don't fossilize. Hosts and symbionts do not coevolve so it is unlikely that a molecular signature would be obvious and it would be difficult to date the association between host genotype and symbiont genotype. Although competition could be strong in stable regions, the selection regimes imposed by environment conditions in stable regions would be less extreme. To distinguish between centers of accumulation in stable vs. unstable regions, it may be possible to compare the rate of evolution in various parts of the genome to distinguish between the two types of selection. In addition, the functional efficiency (for functions related to the tolerance of environmental parameters) of the symbiosis would be more normally distributed in a stable region. This could be compared to a region with unstable conditions where strong, directional selection would skew the distribution toward high functional efficiency. I observed multiple members of clade A symbionts in clams from East Africa. Clade A is the most basal lineage within *Symbiodinium*. It was most likely the first to diverge from free-living ancestors and it is the least efficient symbiont from the perspective of the host (Stat et al. 2008). However the majority of symbionts from the WIO were phylotypes from clade C. The crown group was better represented in both host species in East Africa than in other regions across the IWP. To address center of accumulation hypotheses, for the Central IWP and the WIO, functional diversity for lineages within *Symbiodinium,* the ages of the various holobiont combinations and the prevalence of endemic lineages would need to be tested with comparative methods.

4. Limited niche space and relaxed specificity

The genus *Symbiodinium* (order Suessiales) is genetically diverse and includes what would be considered family level diversity in other orders of dinoflagellates such as Gymnodiniales and Peridiniales (Rowan and Powers 1992). Lineages of *Symbiodinium* are sometimes specific for a particular host but they are often found in a variety of hosts across phyla and depend on geography and/or local ecology (LaJeunesse et al. 2004b; Pochon et al. 2004). Generalist symbionts, like ITS2 type C1, are ancestral lineages from which specialized types are derived (Correa and Baker 2009). Across the IWP, most giant clams hosted generalist symbionts. These lineages were not specific for Tridacnidae and because under horizontal transmission, there would be limited opportunities for strict coevolution (Chapter 1). However a few individual clams hosted symbionts not found in clams from other regions. Although ITS2 type A1 has been found in a diverse group of hosts across the tropics (LaJeunesse et al. 2009), it has only been identified in giant clams from the Red Sea (Chapter 2) suggesting that giant clams from some locations host specific symbiont types. Along the East African coast, most clams hosted ITS2 type C1 but a few individual clams hosted rare alternative lineages.

Fewer hosts means less niche space. If an ecosystem supports limited hosts but diverse symbiont communities, including both specific, host-adapted types and broadly distributed generalist types, symbionts without a specific host either: (1) go extinct in this area, (2) adapt to a new, relatively unpopulated host with low symbiont density, or (3) compete with existing symbionts to share the local host population. Six representatives from three major clades associated with two species of giant clam hosts on East African reefs. All but one of these lineages also associated with hosts on reefs in the central IWP. Initial observations suggested that, far from the center of biodiversity, the stable conditions along the less complex East African coast allowed rare *Symbiodinium* to persist despite the limited niche space available in only two host species.

In complex communities, pathogens evolve virulence and cooperative hosts evolve specificity for high performing symbionts when competing with diverse, proliferating strains, many of which may be cheaters (Arneberg et al. 1998; Doebeli and Knowlton 1998; Thrall et al. 2007; Kiers et al. 2008). The complexity of coral reefs and the prevalence of alternative hosts may be what allowed corals to evolve specific cooperative relationships with mutualistic *Symbiodinium* and other microbes (Thurber et al. 2009). In regions where diverse alternative hosts are available, specific, highly productive holobionts cooperate efficiently, and exclude less productive combinations. Alternatively, in regions of low habitat complexity, where hosts are limited, partners relax specificity because they cannot afford to be choosy. In addition, symbionts are less diverse and cheating is less prevalent; therefore, hosts do not have to enforce sanctions against uncooperative strains (Thrall et al. 2007; Kiers et al. 2008). In regions with limited diversity, *Symbiodinium* may colonize "second choice hosts"; an association that would not be competitive in other, more complex regions. Selection against less efficient partners is relaxed and may not distinguish between the cheaters and efficient cooperators. Thus, the successful persistence of less competitive combinations would inflate holobiont diversity in regions with limited host diversity such as reefs along the East African coast.

In East Africa, diverse symbiont lineages shared two host species. If limited habitat and relaxed specificity explained the additional diversity crowded into few hosts, I would expect to find the rare symbiont lineages from East Africa, specific for certain giant clam species in Papua New Guinea and Australia. In these high diversity regions, symbionts would colonize their first choice host (or vice versa) in the short term and promote coevolution between specific partners in the long term. However, the rare lineages from East Africa, including D1, A3, A3a, and C66, partnered with multiple tridacnid hosts in the Central IWP. And in other regions where host diversity was limited, these symbiont lineages were not present (Chapter 1). These data rejected the hypothesis that lower biotic complexity relaxed host-symbiont specificity and allowed additional symbiont diversity to share the available hosts, thus inflating holobiont diversity. Competition between multiple lineages of *Symbiodinium* contributed to ecological dynamics, even when additional host species provided additional niche space in the Central IWP. Perhaps these lineages of dinoflagellates adapted to the symbiotic lifestyle and can easily reestablish symbioses with multiple hosts. Their lack of specificity within giant clams and their associations with diverse host phyla, outside giant clams, supports the hypothesis that prolonged reciprocal adaptation is not necessary to evolve stable, long-term symbioses (Kiers et al. 2008).

5. Historical legacy of Miocene diversity

Species distributions are a product of modern ecological interactions and historical processes. Models for the evolution of mutualisms, niche theory and the historical geology and oceanography could not satisfactorily explain community diversity patterns in *Symbiodinium* in giant clams. I evaluated historical biogeography of hosts, symbionts and their associations though time which indicated that the high diversity of holobionts along the East African coast is a relict of historical giant clam diversity in the WIO and subsequent range shifts.

The center of diversity for the giant clam lineage has shifted over the last 50 million years. Giant clams first evolved in the warm shallow seas of what is now southern Europe. These lineages shifted east and new lineages appeared in the African – Arabian region before they went extinct in the Tethys and expanded into the Indo-Pacific (Harzhauser et al. 2008). While the holobiont distribution mirrored this shifting host range distribution, the rate of range

shift for communities of associations between host and symbiont was slower than the shift of the individual host lineages. Biodiversity for giant clam hosts was highest in the West Indian Ocean during the Miocene and modern holobiont diversity remains high in this region even though hosts have since gone extinct here as their range shifted east and the center of host biodiversity is now the Central IWP (Harzhauser et al. 2008).

As Tridacnidae diversified and expanded east from the Tethys through the Arabian Shelf, the Indian Ocean and into the Pacific Ocean, centers of biodiversity also shifted east for a variety of other marine taxa including mangroves, foraminifera, gastropods and scleractinian corals (Harzhauser et al. 2007; Williams 2007; Renema et al. 2008). *Symbiodinium* hosting organisms such as corals, forams and clams must have brought their symbiont populations as they colonized new regions because the mutualism is obligate for these hosts. While host distribution shifts are traceable through time because shells and skeletons remain preserved in the fossil record, evidence of historical diversity in *Symbiodinium* must be inferred using sequence data.

Molecular clock interpretation for *Symbiodinium* rRNA sequence data suggested that the emergence and radiation of Clade C *Symbiodinium* began in the Miocene, concurrent with the arrival of members of Tridacnidae and Scleractinia in the Indian Ocean (LaJeunesse 2005; Harzhauser et al. 2007; Harzhauser et al. 2008). Various lineages of clade C *Symbiodinium* were the most prevalent group of symbionts in Indo-Pacific corals (LaJeunesse 2005). They are also found in giant clams (Chapter 1; Baillie et al. 2000). This symbiont lineage was dominant in the *T. maxima* and *T. squamosa* populations that I collected along the East African coastline; clade C phylotypes were found in 84% of the samples from this region including all of the *T. squamosa* individuals. Elsewhere across the giant clam distribution, clade C was less prevalent and only 33% of the clams from the Central IWP hosted symbionts from this clade. ITS2 type C1 forms the center of a cluster, which has since diversified (LaJeunesse 2005; Correa and Baker 2009). This basal phylotype diverged from the *Symbiodinium* stem group in the Miocene when the clam distribution was centered in this region. The C1 lineage subsequently radiated into a diverse crown group and includes many specific lineages including ITS2 type C66, which is not known from giant clams outside these two regions (Chapter 1). The *Symbiodinium* crown group diversified concordant with the giant clam center of biodiversity shift into the Central IWP. These data suggested that giant clams predominantly hosted ancestral C1 symbionts in the WIO because both partners colonized the region at the same time.

Six lines of evidence support the hypothesis that the secondary center of giant clam holobiont diversity in East Africa is a Miocene relict of the African-Arabian center of diversity for *Symbiodinium* hosting marine organisms. (1) Giant clams were most diverse in the Arabian-East African province during the Miocene. (2) The giant clam center of diversity shifted into Central IWP during the Pliocene. (3) Centers of biodiversity for other *Symbiodinium* hosts also shifted through the Arabian-East African province during the Miocene. (4) *Symbiodinium* clade C diverged and diversified during the Miocene. (5) The ancestral *Symbiodinium* phylotype C1 dominated the East African tridacnid populations. And (6) the highest diversity of Tridacnidae holobionts outside of the Central IWP was observed in the WIO population.

The western edge of the holobiont range appears to be shrinking faster than the host range. Fossil *T. gigas* from terraces along the East African coast went locally extinct in the Pliocene (Crame 1986; Harzhauser et al. 2008). Although modern *T. gigas* populations are confined to the Pacific, additional *Symbiondinim* diversity would have supported a diverse Miocene-Pliocene giant clam assemblage. Residual symbiont lineages associated with modern East African *T. maxima* individuals because host diversity is now minimal and host-symbiont

specificity has relaxed. It is possible that the additional symbiont lineage diversity also colonized alternative host organisms such as corals and forams.

The two giant clam species that survived late Pleistocene range reductions and rare, relict *Symbiodinium* populations lingered in unusual host-symbiont combinations although their original hosts are now locally extinct. Additional fossils or other evidence of extinct host lineages from East Africa or Arabia would further support this hypothesis that high holobiont diversity on East African reefs is related to historical host diversity. If the holobiont range is still receding, mirroring the range reduction of the host lineage, in another few million years the East African *T. maxima* and *T. squamosa* will be as symbiont specific as their Pacific island relatives.

I considered sampling bias, abiotic factors, niche dynamics and history to explain a secondary center of diversity for giant clam-*Symbiodinium* holobionts on East African reefs. The availability of continuous continental habitat, environmental stability and host specificity contributed to high holobiont diversity. Historical evidence partially decoupled host from holobiont supporting the idea of holobiont biogeography. The giant clam-*Symbiodinium* holobiont distribution shifted eastward more slowly than the giant clam distribution and modern holobiont diversity in East Africa is a relict of Miocene biodiversity.

CONCLUSIONS

The marine biodiversity gradient peaks in the Central IWP and the number of lineages diminishes to the east and west. However, although this pattern was observed in Tridacnidae, *Symbiodinium* sampled from giant clams were equally diverse in the WIO and the Central IWP. A unique lineage, unknown from giant clams in other regions, was found in a single host individual and several endemic holobionts were described from the WIO.

I examined the origins of an unexpected center of biodiversity in the West Indian Ocean for Tridacnidae-*Symbiodinium* holobionts. The WIO is under-sampled relative to the Central IWP where multiple research efforts have broadly characterized diverse reef taxa. Undescribed lineages native to the WIO may obscure patterns in the global diversity gradient. I also considered the possibility that the continuous continental fringing reefs along the African coast removed the inconsistency associated with dispersal mechanisms. But since other continental fringing reefs outside of the Central IWP were not as diverse as the WIO, I determined that efficient dispersal along continents was not a major process contributing to WIO diversity.

The Central IWP has been tectonically and oceanographically variable over the last 25 million years in contrast to East Africa. The geographic complexity may have contributed to diverse communities in the Central IWP as either a driver of evolution (cradle of diversity) or as a sink for populations emigrating from other regions (museum of diversity) as conditions changed. Regardless of which mechanism(s) generated the pattern, the unstable environment would have selected for only the most tolerant holobionts. Under stable conditions in the WIO, intolerant holobionts would have survived because selection against them was minimal. This would make the WIO a center of accumulation for marginally functional holobionts and I would expect to find unique combinations of hosts and symbionts in the WIO. Because only a few of the holobionts were unknown from other regions I concluded that this process was functioning on a small scale but that more data would be required to further test the hypothesis.

Mutualisms are generally more specific in highly complex regions. I considered the hypothesis that holobionts were diverse in the WIO because niche space was limited to two hosts and relaxed specificity allowed diverse *Symbiodinium* populations to share them. The Central

IWP is diverse both geologically and biologically; however, I did not observe increased symbiont specificity in giant clams from that region. I concluded that a few *Symbiodinium* lineages, such as C66, might share the limited host population because of relaxed specificity in the WIO to but this hypothesis was tentatively rejected because other regions that were host depauperate also lacked symbiont diversity.

I evaluated the historical biogeography for the clams and for *Symbiodinium* as well as the *Symbiodinium*-Tridacnidae holobiont. As the center of biodiversity for tridacnid clams shifted from the Tethys Sea through the African-Arabian province into the IWP over the last 50 million years, *Symbiodinium* clade C simultaneously diverged and diversified. Both species of clam in the WIO hosted clade C symbionts and rare alternative lineages were more common here than in the Pacific Ocean where *T. squamosa* was specific for clade C but *T. maxima* hosted clade A. These data suggested that WIO holobionts are a legacy of the Miocene diversity. As the larger clams went extinct, *Symbiodinium* found refuge in persisting host lineages, inflating holobiont diversity. Historical biogeographic patterns for combinations of hosts and symbionts can provide additional information when the distribution of either partner, geologic, and ecologic hypotheses cannot explain modern holobiont diversity patterns.

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APPENDIX

A.1.1

Tridacna maxima – ITS2 types and locality information

Sample	ITS2 type	Locality	site name	Latitude	Longitude
HG.010	A1	Egypt	Gifton Islands	27.268917	33.8942
HG.011	A1	Egypt	Gifton Islands	27.268917	33.8942
HG.012	A1	Egypt	Gifton Islands	27.268917	33.8942
HG.013	A1	Egypt	Gifton Islands	27.268917	33.8942
HG.014	A1	Egypt	Gifton Islands	27.268917	33.8942
RM.008	A1	Egypt	Ras Nasrani	27.964633	34.415783
RM.009	A1	Egypt	Ras Nasrani	27.964633	34.415783
RM.010	A1	Egypt	Ras Nasrani	27.964633	34.415783
RM.011	A1	Egypt	Ras Nasrani	27.964633	34.415783
RM.012	A1	Egypt	Ras Nasrani	27.964633	34.415783
HG.046	A1	Egypt	Mangrove Bay	25.871833	34.418783
HG.047	A1	Egypt	Mangrove Bay	25.871833	34.418783
HG.048	A1	Egypt	Mangrove Bay	25.871833	34.418783
HG.049	A1	Egypt	Mangrove Bay	25.871833	34.418783
HG.050	A1	Egypt	Mangrove Bay	25.871833	34.418783
DH.003	A1	Egypt	Blue hole	28.557383	34.523833
DH.006	A1	Egypt	Blue hole	28.557383	34.523833
DH.007	A1	Egypt	Blue hole	28.557383	34.523833
DH.008	A1	Egypt	Blue hole	28.557383	34.523833
DH.009	A1	Egypt	Blue hole	28.557383	34.523833
ZO.731	A3	Africa	Zanzibar	-6.027317	39.1316
ZB.011	A3	Africa	Bungalow Islands	-6.0044	39.147117
LU.008	A3	Africa	Adi	-2.0362	40.980083
AT.006	A3	Aitutaki	lagoon	-18.901189	-159.77445
AT.008	A3	Aitutaki	lagoon	-18.901189	-159.77445
AT.141	A3	Aitutaki	boathouse	-18.848383	-159.75423
AT.143	A3	Aitutaki	boathouse	-18.848383	-159.75423
LI.077	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.078	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.079	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.080	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.082	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.083	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.084	A3	Australia	Loomis Pt	-14.686919	145.449469
LI.086	A3	Australia	Loomis Pt	-14.686919	145.449469
VO.015	A3	Fiji	Pola's spot	-17.254283	178.17075
KO.004	A3	Fiji	Koro, Nacamaki	-17.2304	179.439283
KV.011	A3	Kavieng	Nusa Lik	-2.577609	150.771988
AD.049	A3	Maldives	Mudakan	-0.1001	73.16445
AD.055	A3	Maldives	Mudakan	-0.1001	73.16445
ME.030	A3	Maldives	Hans Hass Reef	4.008467	73.454017
ME.032	A3	Maldives	Hans Hass Reef	4.008467	73.454017
ME.033	A3	Maldives	Hans Hass Reef	4.008467	73.454017

NS.002	A3	Moorea	N. Sheraton	-17.47791	-149.81787
WCB.002	A3	Moorea	W. cooks Bay	-17.47791	-149.81787
NO.003	A3	New Cal	Passe Exterieure	-22.495217	166.437583
NO.006	A3	New Cal	Passe Exterieure	-22.495217	166.437583
RT.101	A3	Rarotonga	Saltwater Café	-21.267067	-159.77218
RT.102	A3	Rarotonga	Saltwater Café	-21.267067	-159.77218
RT.105	A3	Rarotonga	Saltwater Café	-21.267067	-159.77218
RT.006	A3	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
RT.008	A3	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
OF.004	A3	Samoa	Pool 400	-14.1776	-169.65422
OF.005	A3	Samoa	Pool 400	-14.1776	-169.65422
OF.006	A3	Samoa	Pool 400	-14.1776	-169.65422
OF.007	A3	Samoa	Pool 400	-14.1776	-169.65422
OF.009	A3	Samoa	Pool 400	-14.1776	-169.65422
OF.010	A3	Samoa	Pool 400	-14.1776	-169.65422
TC.002	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.003	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.006	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.007	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.008	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.009	A3	Sri Lanka	Navy base	8.589902	81.236011
TC.010	A3	Sri Lanka	Navy base	8.589902	81.236011
Ef.001	A3	Vanuatu	Sunae Village	-17.5102	168.298533
EF.002	A3	Vanuatu	Sunae Village	-17.5102	168.298533
EF.003	A3	Vanuatu	Sunae Village	-17.5102	168.298533
EF.011	A3	Vanuatu	Sunae Village	-17.5102	168.298533
EF.013	A3	Vanuatu	Sunae Village	-17.5102	168.298533
EF.015	A3	Vanuatu	Sunae Village	-17.5102	168.298533
LU.004	A3a	Africa	Adi	-2.0362	40.980083
KV.020	A3a	Kavieng	Emago Isl	-2.617486	150.737964
KV.024	A3a	Kavieng	Emago Isl	-2.617486	150.737964
KV.026	A3a	Kavieng	Ral Isl	-2.616477	150.736959
KV.027	A3a	Kavieng	Ral Isl	-2.616477	150.736959
KV.054	A3a	Kavieng	Nusaum	-2.63766	150.639522
KV.060	A3a	Kavieng	Bismark Sea	-2.753159	150.710733
AD.001	A3a	Maldives	Addu, Vilingili	-0.018717	73.182167
AD.003	A3a	Maldives	Addu, Vilingili	-0.018717	73.182167
AD.005	A3a	Maldives	Addu, Vilingili	-0.018717	73.182167
AD.006	A3a	Maldives	Addu, Vilingili	-0.018717	73.182167
AD.036	A3a	Maldives	KudaKandu	-0.110833	73.121633
AD.046	A3a	Maldives	KudaKandu	-0.1001	73.16445
AT.005	A3x	Aitutaki	lagoon	-18.901189	-159.77445
VO.002	A3x	Fiji	Voli Voli	-17.254284	178.17075
NO.004	A3x	New Cal	Passe Exterieure	-22.495217	166.437583
RT.005	A3x	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
OF.001	A3x	Samoa	Pool 400	-14.1776	-169.65422
OF.002	A3x	Samoa	Pool 400	-14.1776	-169.65422
OF.003	A3x	Samoa	Pool 400	-14.1776	-169.65422
OF.008	A3x	Samoa	Pool 400	-14.1776	-169.65422

LU.012	A4	Africa	Adi	-2.0362	40.980083
AT.142	A6	Aitutaki	boathouse	-18.848383	-159.75423
KV.003	A6	Kavieng	Nango Island	-2.577609	150.771988
KV.052	A6	Kavieng	Nusaum	-2.63766	150.639522
KV.062	A6	Kavieng	Bismark Sea	-2.753159	150.710733
ECB.001	A6	Moorea	E. cooks bay	-17.47791	-149.81787
ECB.002	A6	Moorea	E. cooks bay	-17.47791	-149.81787
EPN.001	A6	Moorea	E. Pavement Nui	-17.475911	-149.80749
EPN.002	A6	Moorea	E. Pavement Nui	-17.475911	-149.80749
NS.001	A6	Moorea	N. Sheraton	-17.47791	-149.81787
TM.001	A6	Moorea	Temae	-17.498907	-149.75605
TM.002	A6	Moorea	Temae	-17.498907	-149.75605
TM.003	A6	Moorea	Temae	-17.498907	-149.75605
WCB.001	A6	Moorea	W. cooks Bay	-17.47791	-149.81787
NO.015	A6	New Cal	Passe Interieur	-22.488867	166.439317
NO.016	A6	New Cal	Passe Interieur	-22.488867	166.439317
RT.004	A6	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
RT.009	A6	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
RT.103	A6	Rarotonga	Saltwater Café	-21.267067	-159.77218
TC.001	A6	Sri Lanka	Navy base	8.589902	81.236011
PHI.001	A6	Thailand	Phi Phi	7.723569	98.775689
PHI.003	A6	Thailand	Phi Phi	7.723569	98.775689
PHI.004	A6	Thailand	Phi Phi	7.723569	98.775689
PHI.005	A6	Thailand	Phi Phi	7.723569	98.775689
PHU.001	A6	Thailand	Phuket	7.953637	98.248415
PHU.002	A6	Thailand	Phuket	7.953637	98.248415
PHU.003	A6	Thailand	Phuket	7.953637	98.248415
PHU.004	A6	Thailand	Phuket	7.953637	98.248415
PHU.005	A6	Thailand	Phuket	7.953637	98.248415
LU.002	C1	Africa	Wakuku	-2.043983	40.996367
LU.003	C1	Africa	Adi	-2.0362	40.980083
LU.005	C1	Africa	Adi	-2.0362	40.980083
LU.013	C1	Africa	Adi	-2.0362	40.980083
LU.014	C1	Africa	Adi	-2.0362	40.980083
MB.001	C1	Africa	Ras Iwatine	-4.0178	39.730917
MB.002	C1	Africa	Ras Iwatine	-4.0178	39.730917
MB.004	C1	Africa	Ras Iwatine	-4.0178	39.730917
MB.005	C1	Africa	Ras Iwatine	-4.0178	39.730917
MB.006	C1	Africa	Ras Iwatine	-4.0178	39.730917
ZB.001	C1	Africa	Sand Island	-6.157733	39.133133
ZB.002	C1	Africa	Sand Island	-6.157733	39.133133
ZB.004	C1	Africa	Sand Island	-6.157733	39.133133
ZB.005	C1	Africa	Sand Island	-6.157733	39.133133
ZB.007	C1	Africa	Sand Island	-6.157733	39.133133
ZB.008	C1	Africa	Bungalow Islands	-6.0044	39.147117
ZB.009	C1	Africa	Bungalow Islands	-6.0044	39.147117
ZO.7170	C1	Africa	Zanzibar	-6.027317	39.1316
ZO.7238	C1	Africa	Zanzibar	-6.027317	39.1316
ZO.7239	C1	Africa	Zanzibar	-6.027317	39.1316

BQ.024	C1	Fiji	Beqa Lagoon	-18.324817	178.134717
KO.006	C1	Fiji	Koro, Nacamaki	-17.2304	179.439283
KO.007	C1	Fiji	Koro, Nacamaki	-17.2304	179.439283
KO.008	C1	Fiji	Koro, Nacamaki	-17.2304	179.439283
KO.009	C1	Fiji	Koro, Nacamaki	-17.2304	179.439283
KV.001	C1	Kavieng	Nango Island	-2.577609	150.771988
KV.019	C1	Kavieng	Emago Isl	-2.617486	150.737964
KV.025	C1	Kavieng	Ral Isl	-2.616477	150.736959
KV.059	C1	Kavieng	Bismark Sea	-2.753159	150.710733
AD.023	C1	Maldives	KudaKandu	-0.110833	73.121633
RT.010	C1	Rarotonga	Fruits Rarotonga	-21.2709	-159.74368
EF.006	C1	Vanuatu	Sunae Village	-17.5102	168.298533
EF.010	C1	Vanuatu	Sunae Village	-17.5102	168.298533
EF.012	C1	Vanuatu	Sunae Village	-17.5102	168.298533
EF.016	C1	Vanuatu	Sunae Village	-17.5102	168.298533
LI.085	C2	Australia	Loomis Pt	-14.686919	145.449469
KB.035	C2	Kimbe	Matane Huva	-5.550534	150.15104
KB.036	C2	Kimbe	Matane Huva	-5.550534	150.15104
LU.011	C66	Africa	Adi	-2.0362	40.980083
LU.001	D1	Africa	Wakuku	-2.043983	40.996367
KB.049	D1	Kimbe	Venessa	-5.424067	150.209472
TC.005	D1	Sri Lanka	Navy base	8.589902	81.236011

A.1.2

Tridacna maxima – Reef environment and depth

Sample	ITS2 type	Locality	Reef type	Depth (m)
HG.010	A1	Egypt	patch	5.4864
HG.011	A1	Egypt	patch	6.096
HG.012	A1	Egypt	patch	3.048
HG.013	A1	Egypt	patch	3.6576
HG.014	A1	Egypt	patch	3.048
RM.008	A1	Egypt	fringing	13.1064
RM.009	A1	Egypt	fringing	14.6304
RM.010	A1	Egypt	fringing	11.5824
RM.011	A1	Egypt	fringing	11.8872
RM.012	A1	Egypt	fringing	13.1064
HG.046	A1	Egypt	fringing	12.192
HG.047	A1	Egypt	fringing	12.192
HG.048	A1	Egypt	fringing	10.668
HG.049	A1	Egypt	fringing	9.7536
HG.050	A1	Egypt	fringing	6.4008
DH.003	A1	Egypt	fringing	14.0208
DH.006	A1	Egypt	fringing	10.0584
DH.007	A1	Egypt	fringing	9.7536
DH.008	A1	Egypt	fringing	6.7056
DH.009	A1	Egypt	fringing	4.572
ZO.731	A3	Africa	patch	5.7912
ZB.011	A3	Africa	patch	5.7912
LU.008	A3	Africa	fringing	1.8288
AT.006	A3	Aitutaki	lagoon	0.6096
AT.008	A3	Aitutaki	lagoon	0.9144
AT.141	A3	Aitutaki	lagoon	0.3048
AT.143	A3	Aitutaki	lagoon	0.3048
LI.077	A3	Australia	lagoon	2.1336
LI.078	A3	Australia	lagoon	2.1336
LI.079	A3	Australia	lagoon	2.1336
LI.080	A3	Australia	lagoon	2.1336
LI.082	A3	Australia	lagoon	2.1336
LI.083	A3	Australia	lagoon	2.1336
LI.084	A3	Australia	lagoon	2.1336
LI.086	A3	Australia	lagoon	2.1336
VO.015	A3	Fiji	fringing	12.8016
KO.004	A3	Fiji	fringing	12.8016
KV.011	A3	Kavieng	patch	0.9144
AD.049	A3	Maldives	fringing	17.0688
AD.055	A3	Maldives	fringing	20.7264
ME.030	A3	Maldives	patch	2.4384

ME.032	A3	Maldives	patch	1.8288
ME.033	A3	Maldives	patch	1.8288
NS.002	A3	Moorea	lagoon	2.4384
WCB.002	A3	Moorea	lagoon	1.8288
NO.003	A3	New Cal	fringing	13.1064
NO.006	A3	New Cal	fringing	14.6304
RT.101	A3	Rarotonga	lagoon	1.2192
RT.102	A3	Rarotonga	lagoon	0.9144
RT.105	A3	Rarotonga	lagoon	0.9144
RT.006	A3	Rarotonga	lagoon	2.4384
RT.008	A3	Rarotonga	lagoon	0.9144
OF.004	A3	Samoa	lagoon	1.2192
OF.005	A3	Samoa	lagoon	1.524
OF.006	A3	Samoa	lagoon	0.6096
OF.007	A3	Samoa	lagoon	0.9144
OF.009	A3	Samoa	lagoon	1.2192
OF.010	A3	Samoa	lagoon	0.9144
TC.002	A3	Sri Lanka	fringing	6.096
TC.003	A3	Sri Lanka	fringing	5.7912
TC.006	A3	Sri Lanka	fringing	3.9624
TC.007	A3	Sri Lanka	fringing	3.6576
TC.008	A3	Sri Lanka	fringing	3.9624
TC.009	A3	Sri Lanka	fringing	4.8768
TC.010	A3	Sri Lanka	fringing	5.1816
EF.001	A3	Vanuatu	lagoon	3.048
EF.002	A3	Vanuatu	lagoon	3.048
EF.003	A3	Vanuatu	lagoon	3.048
EF.011	A3	Vanuatu	lagoon	1.8288
EF.013	A3	Vanuatu	lagoon	3.6576
EF.015	A3	Vanuatu	lagoon	2.1336
LU.004	A3a	Africa	fringing	2.4384
KV.020	A3a	Kavieng	patch	3.048
KV.024	A3a	Kavieng	patch	6.096
KV.026	A3a	Kavieng	patch	8.5344
KV.027	A3a	Kavieng	patch	8.5344
KV.054	A3a	Kavieng	patch	8.8392
KV.060	A3a	Kavieng	fringing	3.9624
AD.001	A3a	Maldives	fringing	7.62
AD.003	A3a	Maldives	fringing	7.0104
AD.005	A3a	Maldives	fringing	10.9728
AD.006	A3a	Maldives	fringing	10.0584
AD.036	A3a	Maldives	fringing	19.812
AD.046	A3a	Maldives	fringing	17.3736
AT.005	A3x	Aitutaki	lagoon	0.6096
VO.002	A3x	Fiji	fringing	4.572
NO.004	A3x	New Cal	fringing	13.1064
RT.005	A3x	Rarotonga	lagoon	1.8288
OF.001	A3x	Samoa	lagoon	0.9144
OF.002	A3x	Samoa	lagoon	1.2192

OF.003	A3x	Samoa	lagoon	1.524
OF.008	A3x	Samoa	lagoon	1.2192
LU.012	A4	Africa	fringing	1.8288
AT.142	A6	Aitutaki	lagoon	0.3048
KV.003	A6	Kavieng	patch	6.7056
KV.052	A6	Kavieng	patch	4.2672
KV.062	A6	Kavieng	fringing	2.7432
ECB.001	A6	Moorea	lagoon	2.1336
ECB.002	A6	Moorea	lagoon	1.2192
EPN.001	A6	Moorea	lagoon	1.8288
EPN.002	A6	Moorea	lagoon	1.8288
NS.001	A6	Moorea	lagoon	1.8288
TM.001	A6	Moorea	lagoon	1.2192
TM.002	A6	Moorea	lagoon	1.524
TM.003	A6	Moorea	lagoon	1.2192
WCB.001	A6	Moorea	lagoon	1.8288
NO.015	A6	New Cal	fringing	11.5824
NO.016	A6	New Cal	fringing	11.5824
RT.004	A6	Rarotonga	lagoon	0.6096
RT.009	A6	Rarotonga	lagoon	0.3048
RT.103	A6	Rarotonga	lagoon	0.9144
TC.001	A6	Sri Lanka	fringing	6.096
PHI.001	A6	Thailand	unknown	unknown
PHI.003	A6	Thailand	unknown	unknown
PHI.004	A6	Thailand	unknown	unknown
PHI.005	A6	Thailand	unknown	unknown
PHU.001	A6	Thailand	unknown	unknown
PHU.002	A6	Thailand	unknown	unknown
PHU.003	A6	Thailand	unknown	unknown
PHU.004	A6	Thailand	unknown	unknown
PHU.005	A6	Thailand	unknown	unknown
LU.002	C1	Africa	fringing	0.9144
LU.003	C1	Africa	fringing	0.6096
LU.005	C1	Africa	fringing	0.9144
LU.013	C1	Africa	fringing	1.2192
LU.014	C1	Africa	fringing	0.9144
MB.001	C1	Africa	lagoon	0.4572
MB.002	C1	Africa	lagoon	0.4572
MB.004	C1	Africa	lagoon	0.4572
MB.005	C1	Africa	lagoon	0.4572
MB.006	C1	Africa	lagoon	0.4572
ZB.001	C1	Africa	patch	10.9728
ZB.002	C1	Africa	patch	10.3632
ZB.004	C1	Africa	patch	10.3632
ZB.005	C1	Africa	patch	9.7536
ZB.007	C1	Africa	patch	4.572
ZB.008	C1	Africa	patch	10.0584
ZB.009	C1	Africa	patch	3.3528
ZO.7170	C1	Africa	patch	unknown

ZO.7238	C1	Africa	patch	unknown
ZO.7239	C1	Africa	patch	unknown
BQ.024	C1	Fiji	patch	3.048
KO.006	C1	Fiji	fringing	7.0104
KO.007	C1	Fiji	fringing	6.7056
KO.008	C1	Fiji	fringing	7.3152
KO.009	C1	Fiji	fringing	7.3152
KV.001	C1	Kavieng	patch	5.1816
KV.019	C1	Kavieng	patch	3.048
KV.025	C1	Kavieng	patch	6.096
KV.059	C1	Kavieng	fringing	19.5072
AD.023	C1	Maldives	fringing	18.288
RT.010	C1	Rarotonga	lagoon	1.8288
EF.006	C1	Vanuatu	lagoon	1.2192
EF.010	C1	Vanuatu	lagoon	1.8288
EF.012	C1	Vanuatu	lagoon	2.7432
EF.016	C1	Vanuatu	lagoon	3.3528
LI.085	C2	Australia	lagoon	2.1336
KB.035	C2	Kimbe	patch	3.9624
KB.036	C2	Kimbe	patch	3.9624
LU.011	C66	Africa	fringing	0.6096
LU.001	D1	Africa	fringing	0.9144
KB.049	D1	Kimbe	patch	1.524
TC.005	D1	Sri Lanka	fringing	4.2672

A.1.3

Tridacna squamosa – ITS2 types and locality information

Sample	ITS2 type	Locality	Site name	Latitude	Longitude
DH.011	A1	Egypt	Ricks Reef	28.557383	34.523833
HG.009	A1	Egypt	Gifton Islands	27.268917	33.8942
BQ.005	A3	Fiji	Beqa Lagoon	-18.324817	178.134717
ME.004	A3	Maldives	S. Male Atoll	4.110835	73.47428
ME.013	A3	Maldives	S. Male Atoll	4.110835	73.47428
KV.047	A3a	Kavieng	Enuk Island	-2.651323	150.721404
KV.050	A3a	Kavieng	Lemos	-2.63766	150.639522
KV.018	A3x	Kavieng	Emago Isl	-2.617486	150.737964
KV.033	A3x	Kavieng	Enuk Island	-2.651323	150.721404
MB.011	C1	Africa	Ras Iwatine	-4.0178	39.730917
ZB.003	C1	Africa	Sand Island	-6.157733	39.133133
ZB.010	C1	Africa	Bungalow Islands	-6.0044	39.147117
ZB.016	C1	Africa	Bungalow Islands	-6.0044	39.147117
ZB.017	C1	Africa	Pinnacles	-6.193983	39.1316
ZB.018	C1	Africa	Pinnacles	-6.193983	39.1316
ZB.019	C1	Africa	Pinnacles	-6.193983	39.1316
ZB.020	C1	Africa	Pinnacles	-6.193983	39.1316
ZO.7237	C1	Africa	Zanzibar	-6.027317	-6.027317
LI.053	C1	Australia	NW Bird Island	-14.689989	145.462605
LI.105	C1	Australia	NW Bird Island	-14.689989	145.462605
DH.004	C1	Egypt	Blue hole	28.557383	34.523833
DH.005	C1	Egypt	Blue hole	28.557383	34.523833
DH.015	C1	Egypt	Ricks Reef	28.557383	34.523833
HG.005	C1	Egypt	Gifton Islands	27.268917	33.8942
HG.006	C1	Egypt	Gifton Islands	27.268917	33.8942
HG.030	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.032	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.033	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.034	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.035	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.053	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.055	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.059	C1	Egypt	Mangrove Bay	25.871833	34.418783
HG.060	C1	Egypt	Mangrove Bay	25.871833	34.418783
RM.007	C1	Egypt	Ras Nasrani	27.964633	34.415783
BQ.004	C1	Fiji	Beqa Lagoon	-18.324817	178.134717
BQ.007	C1	Fiji	Beqa Lagoon	-18.324817	178.134717
KO.003	C1	Fiji	Koro, Nacamaki	-17.2304	179.439283
KO.025	C1	Fiji	Koro, Vito's Point	-17.241733	179.343583
KO.027	C1	Fiji	Koro, Vito's Point	-17.241733	179.343583
VO.003	C1	Fiji	Voli Voli	-17.254284	178.17075
VO.004	C1	Fiji	Voli Voli	-17.254284	178.17075

VO.005	C1	Fiji	Voli Voli	-17.254284	178.17075
VO.007	C1	Fiji	Voli Voli	-17.254284	178.17075
KB.041	C1	Kimbe	Venessa	-5.424067	150.209472
AD.004	C1	Maldives	Addu, Vilingili	-0.018717	73.182167
AD.028	C1	Maldives	KudaKandu	-0.110833	73.121633
AD.054	C1	Maldives	Mudakan	-0.1001	73.16445
AD.056	C1	Maldives	Mudakan	-0.1001	73.16445
ME.022	C1	Maldives	S. Male Atoll	4.110835	73.47428
ME.026	C1	Maldives	S. Male Atoll	4.110835	73.47428
N0.001	C1	New Cal	Passe Exterieure	-22.495217	166.437583
NO.008	C1	New Cal	Passe Exterieure	-22.495217	166.437583
NO.012	C1	New Cal	Passe Exterieure	-22.495217	166.437583
NO.017	C1	New Cal	Passe Exterieure	-22.495217	166.437583
KV.039	D1	Kavieng	Enuk Island	-2.651323	150.721404
KV.040	D1	Kavieng	Enuk Island	-2.651323	150.721404
KV.057	D1	Kavieng	Bismark Sea	-2.753159	150.710733
KB.001	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.002	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.003	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.005	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.006	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.007	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.023	D1	Kimbe	Matane Huva	-5.550534	150.15104
KB.037	D1	Kimbe	Venessa	-5.424067	150.209472
KB.047	D1	Kimbe	Venessa	-5.424067	150.209472
ME.019	D1	Maldives	S. Male Atoll	4.110835	73.47428

A.1.4

Tridacna squamosa – Reef environment and depth

Sample	ITS2 type	Locality	Reef type	Depth (m)
DH.011	A1	Egypt	fringing	15.24
HG.009	A1	Egypt	patch	9.144
BQ.005	A3	Fiji	patch	10.668
ME.004	A3	Maldives	patch	6.096
ME.013	A3	Maldives	patch	9.4488
KV.047	A3a	Kavieng	patch	13.1064
KV.050	A3a	Kavieng	patch	0.6096
KV.018	A3x	Kavieng	patch	4.8768
KV.033	A3x	Kavieng	patch	1.8288
MB.011	C1	Africa	lagoon	0.9144
ZB.003	C1	Africa	patch	9.7536
ZB.010	C1	Africa	patch	6.4008
ZB.016	C1	Africa	patch	10.9728
ZB.017	C1	Africa	patch	10.9728
ZB.018	C1	Africa	patch	11.2776
ZB.019	C1	Africa	patch	11.2776
ZB.020	C1	Africa	patch	12.192
ZO.7237	C1	Africa	patch	unknown
LI.053	C1	Australia	lagoon	1.524
LI.105	C1	Australia	lagoon	1.524
DH.004	C1	Egypt	fringing	16.764
DH.005	C1	Egypt	fringing	10.0584
DH.015	C1	Egypt	fringing	12.192
HG.005	C1	Egypt	patch	9.7536
HG.006	C1	Egypt	patch	9.4488
HG.030	C1	Egypt	fringing	13.1064
HG.032	C1	Egypt	fringing	20.7264
HG.033	C1	Egypt	fringing	21.336
HG.034	C1	Egypt	fringing	17.0688
HG.035	C1	Egypt	fringing	18.288
HG.053	C1	Egypt	fringing	14.0208
HG.055	C1	Egypt	fringing	9.144
HG.059	C1	Egypt	fringing	9.4488
HG.060	C1	Egypt	fringing	8.5344
RM.007	C1	Egypt	fringing	16.4592
BQ.004	C1	Fiji	patch	10.0584
BQ.007	C1	Fiji	patch	6.7056
KO.003	C1	Fiji	fringing	13.4112
KO.025	C1	Fiji	fringing	9.7536
KO.027	C1	Fiji	fringing	14.6304
VO.003	C1	Fiji	fringing	8.5344
VO.004	C1	Fiji	fringing	7.62

VO.005	C1	Fiji	fringing	14.9352
VO.007	C1	Fiji	fringing	15.5448
KB.041	C1	Kimbe	patch	16.4592
AD.004	C1	Maldives	fringing	9.144
AD.028	C1	Maldives	fringing	9.7536
AD.054	C1	Maldives	fringing	6.096
AD.056	C1	Maldives	fringe	18.8976
ME.022	C1	Maldives	patch	13.1064
ME.026	C1	Maldives	patch	10.3632
N0.001	C1	New Cal	fringe	2.4384
NO.008	C1	New Cal	fringe	8.2296
NO.012	C1	New Cal	fringe	12.4968
NO.017	C1	New Cal	fringe	7.9248
KV.039	D1	Kavieng	patch	1.8288
KV.040	D1	Kavieng	patch	1.8288
KV.057	D1	Kavieng	fringe	17.3736
KB.001	D1	Kimbe	patch	13.716
KB.002	D1	Kimbe	patch	12.192
KB.003	D1	Kimbe	patch	15.8496
KB.005	D1	Kimbe	patch	10.668
KB.006	D1	Kimbe	patch	7.3152
KB.007	D1	Kimbe	patch	4.572
KB.023	D1	Kimbe	patch	9.7536
KB.037	D1	Kimbe	patch	9.7536
KB.047	D1	Kimbe	patch	20.4216
ME.019	D1	Maldives	patch	7.3152

A.3.1
Central Indo West Pacific – Diversity and locality data

Sample	ITS2 type	host	Locality	Latitude	Longitude
LI.010	A3	*Hippopus*	Australia	-14.680062	145.470723
LI.015	A3	*T. crocea*	Australia	-14.66271	145.451838
LI.016	A3	*T. crocea*	Australia	-14.66271	145.451838
LI.017	A3	*T. crocea*	Australia	-14.66271	145.451838
LI.019	A3	*T. crocea*	Australia	-14.66271	145.451838
KV.013	A3	*T. crocea*	Kavieng	-2.577609	150.771988
LI.027	A3	*T. derasa*	Australia	-14.66271	145.451838
LI.048	A3	*T. gigas*	Australia	-14.689989	145.462605
KV.042	A3	*T. gigas*	Kavieng	-2.651323	150.721404
LI.077	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.078	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.079	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.080	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.082	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.083	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.084	A3	*T. maxima*	Australia	-14.686919	145.449469
LI.086	A3	*T. maxima*	Australia	-14.686919	145.449469
KV.011	A3	*T. maxima*	Kavieng	-2.577609	150.771988
KV.020	A3a	*T. maxima*	Kavieng	-2.617486	150.737964
KV.024	A3a	*T. maxima*	Kavieng	-2.617486	150.737964
KV.026	A3a	*T. maxima*	Kavieng	-2.616477	150.736959
KV.027	A3a	*T. maxima*	Kavieng	-2.616477	150.736959
KV.054	A3a	*T. maxima*	Kavieng	-2.63766	150.639522
KV.060	A3a	*T. maxima*	Kavieng	-2.753159	150.710733
KV.047	A3a	*T. squamosa*	Kavieng	-2.651323	150.721404
KV.050	A3a	*T. squamosa*	Kavieng	-2.63766	150.639522
KV.018	A3x	*T. squamosa*	Kavieng	-2.617486	150.737964
KV.033	A3x	*T. squamosa*	Kavieng	-2.651323	150.721404
KV.003	A6	*T. maxima*	Kavieng	-2.577609	150.771988
KV.052	A6	*T. maxima*	Kavieng	-2.63766	150.639522
KV.062	A6	*T. maxima*	Kavieng	-2.753159	150.710733
KV.005	C1	*T. crocea*	Kavieng	-2.577609	150.771988
LI.024	C1	*T. derasa*	Australia	-14.66271	145.451838
LI.030	C1	*T. gigas*	Australia	-14.680062	145.470723
LI.032	C1	*T. gigas*	Australia	-14.680062	145.470723
LI.047	C1	*T. gigas*	Australia	-14.689989	145.462605
LI.050	C1	*T. gigas*	Australia	-14.689989	145.462605
LI.051	C1	*T. gigas*	Australia	-14.689989	145.462605
LI.052	C1	*T. gigas*	Australia	-14.689989	145.462605
KV.002	C1	*T. gigas*	Kavieng	-2.577609	150.771988
KV.034	C1	*T. gigas*	Kavieng	-2.651323	150.721404
KV.045	C1	*T. gigas*	Kavieng	-2.651323	150.721404

KV.056	C1	*T. gigas*	Kavieng	-2.753159	150.710733
KV.001	C1	*T. maxima*	Kavieng	-2.577609	150.771988
KV.019	C1	*T. maxima*	Kavieng	-2.617486	150.737964
KV.025	C1	*T. maxima*	Kavieng	-2.616477	150.736959
KV.059	C1	*T. maxima*	Kavieng	-2.753159	150.710733
LI.053	C1	*T. squamosa*	Australia	-14.689989	145.462605
LI.105	C1	*T. squamosa*	Australia	-14.689989	145.462605
KB.041	C1	*T. squamosa*	Kimbe	-5.424067	150.209472
LI.011	C2	*Hippopus*	Australia	-14.680062	145.470723
LI.012	C2	*Hippopus*	Australia	-14.680062	145.470723
KV.004	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KV.006	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KV.014	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KV.015	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KV.016	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KV.017	C2	*T. crocea*	Kavieng	-2.577609	150.771988
KB.008	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.009	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.012	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.013	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.016	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.018	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.019	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.020	C2	*T. crocea*	Kimbe	-5.550534	150.15104
KB.024	C2	*T. crocea*	Kimbe	-5.550534	150.15104
LI.098	C2	*T. derasa*	Australia	-14.689989	145.462605
KV.043	C2	*T. gigas*	Kavieng	-2.651323	150.721404
KV.046	C2	*T. gigas*	Kavieng	-2.651323	150.721404
LI.085	C2	*T. maxima*	Australia	-14.686919	145.449469
KB.035	C2	*T. maxima*	Kimbe	-5.550534	150.15104
KB.036	C2	*T. maxima*	Kimbe	-5.550534	150.15104
LI.021	C66	*T. crocea*	Australia	-14.66271	145.451838
KV.032	C66	*T. gigas*	Kavieng	-2.651323	150.721404
KV.044	C66	*T. gigas*	Kavieng	-2.651323	150.721404
KB.049	D1	*T. maxima*	Kimbe	-5.424067	150.209472
KV.039	D1	*T. squamosa*	Kavieng	-2.651323	150.721404
KV.040	D1	*T. squamosa*	Kavieng	-2.651323	150.721404
KV.057	D1	*T. squamosa*	Kavieng	-2.753159	150.710733
KB.001	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.002	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.003	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.005	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.006	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.007	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.023	D1	*T. squamosa*	Kimbe	-5.550534	150.15104
KB.037	D1	*T. squamosa*	Kimbe	-5.424067	150.209472
KB.047	D1	*T. squamosa*	Kimbe	-5.424067	150.209472

A.3.2
West Indian Ocean – Diversity and locality data

Sample	ITS2 type	host	Locality	Latitude	Longitude
LU.008	A3	*T. maxima*	Africa	-2.0362	40.980083
ZB.011	A3	*T. maxima*	Africa	-6.0044	39.147117
ZO.731	A3	*T. maxima*	Africa	-6.027317	39.1316
LU.004	A3a	*T. maxima*	Africa	-2.0362	40.980083
LU.012	A4	*T. maxima*	Africa	-2.0362	40.980083
LU.002	C1	*T. maxima*	Africa	-2.043983	40.996367
LU.003	C1	*T. maxima*	Africa	-2.0362	40.980083
LU.005	C1	*T. maxima*	Africa	-2.0362	40.980083
LU.013	C1	*T. maxima*	Africa	-2.0362	40.980083
LU.014	C1	*T. maxima*	Africa	-2.0362	40.980083
MB.001	C1	*T. maxima*	Africa	-4.0178	39.730917
MB.002	C1	*T. maxima*	Africa	-4.0178	39.730917
MB.004	C1	*T. maxima*	Africa	-4.0178	39.730917
MB.005	C1	*T. maxima*	Africa	-4.0178	39.730917
MB.006	C1	*T. maxima*	Africa	-4.0178	39.730917
ZB.001	C1	*T. maxima*	Africa	-6.157733	39.133133
ZB.002	C1	*T. maxima*	Africa	-6.157733	39.133133
ZB.004	C1	*T. maxima*	Africa	-6.157733	39.133133
ZB.005	C1	*T. maxima*	Africa	-6.157733	39.133133
ZB.007	C1	*T. maxima*	Africa	-6.157733	39.133133
ZB.008	C1	*T. maxima*	Africa	-6.0044	39.147117
ZB.009	C1	*T. maxima*	Africa	-6.0044	39.147117
ZO.7170	C1	*T. maxima*	Africa	-6.027317	39.1316
ZO.7238	C1	*T. maxima*	Africa	-6.027317	39.1316
ZO.7239	C1	*T. maxima*	Africa	-6.027317	39.1316
MB.011	C1	*T. squamosa*	Africa	-4.0178	39.730917
ZB.003	C1	*T. squamosa*	Africa	-6.157733	39.133133
ZB.010	C1	*T. squamosa*	Africa	-6.0044	39.147117
ZB.016	C1	*T. squamosa*	Africa	-6.0044	39.147117
ZB.017	C1	*T. squamosa*	Africa	-6.193983	39.1316
ZB.018	C1	*T. squamosa*	Africa	-6.193983	39.1316
ZB.019	C1	*T. squamosa*	Africa	-6.193983	39.1316
ZB.020	C1	*T. squamosa*	Africa	-6.193983	39.1316
ZO.7237	C1	*T. squamosa*	Africa	-6.027317	-6.027317
LU.011	C66	*T. maxima*	Africa	-2.0362	40.980083
LU.001	D1	*T. maxima*	Africa	-2.043983	40.996367